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EFFECT OF SPRAY IRRIGATION OF MUNICIPAL WASTEWATER ON NITROGEN TRANSFORMATIONS IN SOIL

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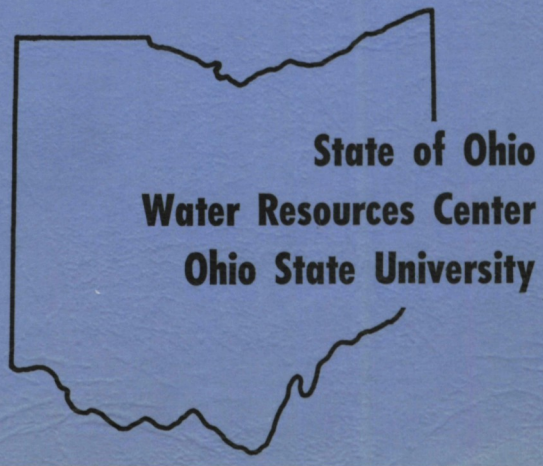

State of Ohio
Water Resources Center
Ohio State University

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by

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ABSTRACT

The overall objective of this research was to respond to the obvious need for more information on nitrogen mineralization and denitrification in soils receiving secondary treated wastewater. The soil-vegetation systems of the Pennsylvania State University Wastewater Management areas (Reed Canarygrass, Old Field, Hardwood Forest and Gameland) used throughout the study. The use of these soils provided a unique opportunity, since the extensive background research data and experiences on these research sites during the past decade were available to assist us in the experimental design and in data interpretations.

One parameter necessary for the calculation of soil nitrogen balances in soils receiving wastewater is nitrogen mineralized from soil organic nitrogen. Plots of nitrogen mineralized using the Stanford and Smith (1972) long term incubation method vs the square root of the time of incubation ($t^{\frac{1}{2}}$) were linear for soils from the 0-7.5 cm depth of all the soil vegetation systems over the entire 26 week incubation period, but only for eight weeks for soils from the 7.5-15 cm depth. This lack of continued mineralization in this soil zone undoubtedly reflects the depletion of mineralizeable organic N compounds early in the incubation period.

Nitrogen mineralization in the wastewater treated soils was generally higher than the control soils and represented about 3.0-3.5% of the pool of soil organic N in all vegetation systems on the Hublersburg soil series. Mineralization of nitrogen was about 5-9 times greater in the coarse textured Morrison soil from the Gameland area and represented about 8.0% of the soil organic N in this soil. This high N mineralization rate could contribute excess nitrogen capable of leaching downward into groundwater. The possibility that organic N from sites on coarse textured soils mineralizes more rapidly should be investigated since it would make these soils less useful for renovation than previously assumed.

Nitrification was found to be rapid in all wastewater treated soils incubated at a variety of temperatures. Nitrification did not occur in the control soils of the Old Field or Gameland sites and was slow in the Hardwood Forest area. This lack of nitrification can readily be explained by the low pH of the control areas compared to the wastewater treated areas. Population estimates of nitrifying bacteria using an MPN method were made but showed no relationship to actual rates of nitrification.

Denitrification losses have generally been assumed to be higher in wastewater treated soils than normal agricultural soils because the higher hydraulic loading associated with wastewater treatment keeps the soil saturated for longer time periods. Denitrification potential measurements in this study have provided evidence that this assumption may not be true. Soils from all of the experimental areas had little denitrifying activity regardless of season and depth, and in both the wastewater treated and control soils. Low denitrifying activity was

expected in soil samples below 15 cm because of a low organic carbon content of these soils, but not in the surface soil zone. Rapid denitrification in both surface soils and those from lower depths when amended with glucose suggests that a lack of available carbon was limiting denitrification. A comparison of the quantities of soluble carbon and mineralizable carbon found in the experimental soils with data reported by Burford and Bremner (1975) supported this conclusion. Plant residues from the various types of vegetation on the Penn State University Wastewater Management area were found to supply available carbon for denitrification for about two to four weeks after incorporation. These annual additions of carbonaceous residue probably enhance denitrification during the spring before plant growth commences, but the effect will be of short duration and of limited value to total nitrogen renovation of applied wastewater.

Measurements were made of the seasonal differences in total root mass and root organic matter, organic carbon, and organic nitrogen in the wastewater treated Reed Canarygrass area during 1974-75. Definite seasonal changes in all parameters were evident with a maximum in summer during the period of maximum growth and a decrease to a minimum in spring before active growth begins. The decrease in root mass, organic matter and organic carbon from July till April would indicate a root turnover rate of about 44-48%. The change in root organic nitrogen from July to April is much less and represents only a 25% decrease.

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INTRODUCTION

Renovation of municipal wastewater by application to soils is a significant alternative to the more conventional physical, chemical, or biological methods for tertiary waste treatment. The numerous symposia and conferences on this land renovation of wastewaters during the past decade attest to the interest which has been generated.

Nitrogen transformations of wastewater nitrogen are of utmost significance to the success of land renovation systems. Failure to achieve the desired degree of nitrogen removal would result in the accumulation of nitrate nitrogen in tile lines and ground water supplies at levels above water quality standards. In essence, the soil system would have failed to provide the desired degree of wastewater treatment. Excess nitrogen in groundwater or tile drainage represents a major potential problem to the success of land renovation for wastewaters.

Nitrogen uptake by vegetation and biological denitrification are the most important processes for the removal of nitrogen from wastewater applied to soils. Adequate nitrogen renovation by vegetation depends upon the ability of the particular plants to adsorb and utilize nitrogen applied with the wastewater. Maximum nitrogen renovation normally occurs when a large portion of the vegetation is removed from the site, e.g. corn silage, forages, etc. However, some vegetation systems seem to operate reasonably well without removal of accumulated vegetation. For example, the hardwood forest and old field sites at the Pennsylvania State Univesity Wastewater Management area have done an excellent job of nitrogen renovation although we know little about the reason for this success. With both types of vegetation, we need to know more about the nitrogen immobilized within the non-harvested portions; whether roots, rhizomes, litter, or residue; and the factors which influence the decomposition of these residues, and the mineralization of the associated nitrogen. We also know relatively little about the magnitude of the root mass associated with a perennial grass, such as Reed Canarygrass, being irrigated with watewater. Seasonal cyclic increases and decreases in root mass should have an effect on the nitrogen pool in the soil, as well as a source of carbon to support biological denitrification.

The significance of biological denitrification to nitrogen renovation in soils receiving wastewater has been difficult to estimate. Biological denitrification is thought to be the major mechanism for removal of any nitrogen in excess of that utilized by the vegetation and particularly for nitrate leached beyond the root zone. Assuming nitrification continues in soils receiving wastewater; high moisture conditions and the possibility of intermittent soil saturation would presumably favor oxygen depletion and increased denitrification. We do not know, however, if the wastewater applied to soils provides sufficient decomposable carbonaceous substrate to support rapid biological denitrification. If not, the heterotrophic denitrifying bacteria must depend upon root exudates, litter, or decomposing plant residues to supply carbon. Likewise, we do not know if sufficient soluble organic matter moves to lower soil horizons to denitrify nitrate leached beyond the root zone into these lower horizons.

The preceeding paragraphs have delineated some of the existing gaps in our knowledge of nitrogen transformation in soils receiving wastewater. The objectives of this research was to respond to the need for more information on nitrogen mineralization and denitrification in soils receiving wastewater. The soil-vegetation systems of the Pennsylvania State University Wastewater Management area used for this study provided unique opportunity to develop a research effort to build upon

and draw from the vast experiences and extensive data already accumulated during the past decade at this site to assist in our experimental design and data interpretation.

MATERIALS AND METHODS

Soils

All soil samples were obtained from the Pennsylvania State University Wastewater Management area. Samples were collected from three vegetation sites (Reed Canarygrass, Old Field, and Hardwood Forest) located on the Hublersburg soil series (Alfic Normudult). These areas have received chlorinated secondary treated wastewater continuously since 1963. A fourth location was a Hardwood forested area on a Morrison sandy loam soil (Ultic Hapludult) which received wastewater applications between 1967-1974. Detailed descriptions and characterization of the soils, underlying bedrock , formations, physiography, and the previous history of the Pennsylvania Wastewater Management area are found in reports by Parizek, et.al (1967) and Kardos, et.al (1974).

The locations of the specific sampling sites are given in Appendix A.

Sampling and Soil Analyses

Soil samples were collected at periodic intervals beginning in August, 1974 and ending on March, 1976. Sampling times coincided with the Summer, Autumn, and Spring seasons of these respective years. Two sampling procedures were followed depending upon specific research needs: Composited 2.5 cm diam.probe samples from 0-7.5, 2.5-15, 15-30, and 30-60 cm and bulk samples from the 0-15 cm depth using a spade.

All soil samples were returned to the laboratories at Ohio State University, mixed and passed through a 2 mm mesh sieve, then stored in plastic bags at 4° C until used for further analyses. All population estimates of nitrifying and denitrifying bacteria were begun within 3 days of sample collection. All other incubations were begun within a 2 week period after sampling.

Physical and chemical data for the experimental soils are summarized in Tables 1, 2, and 3. Organic carbon determinations were by dry combustion, and organic nitrogen by microkjeldahl using a selenium catalyst.

Population Estimates (Nitrifying and Denitrifying Bacteria)

Numbers of nitrifying bacteria within the soil samples were estimated using a Most Probable Number (MPN) technique using the mineral salts medium described by Alexander & Clark (1965). Five replicate tubes per dilution and four 10 fold dilutions were employed. The presence of nitrate after a 30 day incubation at 25° C was determined using Bray's Nitrate Powder (Focht & Joseph, 1973).

Numbers of denitrifying bacteria with the soil samples were estimated using the MPN technique of Focht & Joseph (1973). Five replicate tubes of nitrate broth per dilution were inoculated with four, 10 fold soil dilutions and incubated for 14 days. The presence or absence of nitrate was estimated using Bray's Nitrate Powder.

Table 1. Distribution of Soil Separates in Soils From the Different Vegetation Systems

Vegetation System	Depth	Sand	Silt	Clay	Texture
	cm		%		
Reed Canarygrass	0-7.5	25.3	52.8	21.9	silt loam
	7.5-15	26.9	53.2	19.9	silt loam
	15-30	15.0	48.9	36.1	silty clay loam
	30-60	13.8	46.1	40.1	silty clay loam
Hardwood Forest	0-7.5	41.9	42.3	15.8	loam
	7.5-15	34.2	48.4	17.4	silt loam
	15-30	28.7	48.4	22.9	clay loam
	30-60	32.1	46.0	21.9	clay loam
Old Field	0-7.5	26.9	41.5	31.6	clay loam
	7.5-15	23.7	38.4	37.9	clay loam
	15-30	18.5	39.7	41.8	silty clay
	30-60	20.2	36.3	43.5	clay
Gameland	0-7.5	70.1	22.2	7.7	sandy loam
	7.5-15	70.3	22.7	7.0	sandy loam
	15-30	65.6	24.6	9.8	sandy loam
	30-60	62.6	21.9	15.5	sandy loam

Table 2. Some Selected Chemical Data For Control and Wastewater Treated Sites*

Soil Vegetation	Depth cm	Wastewater Treated Sites pH	Organic C %	Organic N %	C:N	Control Sites pH	Organic C %	Organic N %	C:N
Reed Canarygrass	0-7.5	6.43	3.11	0.255	12.2	6.02	1.83	0.232	7.9
	7.5-15	6.43	1.60	0.132	12.1	5.91	1.19	0.130	9.2
	15-30	6.48	0.98	0.092	10.6	5.65	0.49	0.074	6.6
	30-60	6.17	0.63	0.064	9.8	5.38	0.23	0.030	7.7
Hardwood Forest	0-7.5	6.11	6.39	0.299	21.4	5.90	2.62	0.168	15.6
	7.5-15	5.54	2.00	0.099	20.2	5.24	1.54	0.112	13.8
	15-30	5.22	0.62	0.050	12.4	5.12	1.42	0.065	21.8
	30-60	5.19	0.43	0.042	10.2	5.25	0.78	0.065	11.7
Old Field	0-7.5	6.51	3.39	0.292	11.6	5.11	1.39	0.141	9.9
	7.5-15	6.40	1.05	0.095	11.1	5.02	0.73	0.070	10.4
	15-30	5.96	0.48	0.077	6.2	5.08	0.20	0.034	5.9
	30-60	5.74	0.23	0.064	3.6	5.18	0.14	0.020	7.0
Gameland	0-7.5	6.90	10.69	0.476	22.5	4.74	2.09	0.185	11.3
	7.5-15	6.40	0.96	0.042	22.9	4.98	0.64	0.044	14.5
	15-30	6.16	0.35	0.024	14.6	4.85	0.26	0.040	6.5
	30-60	5.66	0.14	0.018	7.8	4.77	0.20	0.033	6.1

*These data represent means for soil sub-samples collected in July and October (74) and April (75).

Table 3. Some Selected Chemical and Physical Data for Control and Wastewater Treated Sites (0-15 cm depth)

Soil Vegetation	Wastewater Treated Sites						Control Sites					
	pH	Organic C	Organic N	C:N	Moisture Tension 1/3 atm.	Moisture Tension 15 atm	pH	Organic C	Organic N	C:N	Moisture Tension 1/3 atm.	Moisture Tension 15 atm.
		%	%		% H_2O			%	%		% H_2O	
Reed Canarygrass	6.42	2.48	0.266	9.3	29.3	14.3	5.97	1.65	0.197	8.4	23.7	12.8
Hardwood Forest	6.12	3.46	0.263	13.2	37.1	17.6	5.73	5.54	0.190	29.2	23.5	10.9
Old Field	6.46	3.32	0.241	13.8	28.2	15.4	5.06	2.24	0.138	16.2	26.6	10.1
Gameland	6.70	2.78	0.158	17.6	18.9	7.2	4.92	1.00	0.117	8.5	17.1	7.9

Nitrogen Mineralization Studies

Nitrogen mineralization with time from selected soil samples was determined by two methods. Samples collected in July, 1974 from the 0-7.5 & 7.5-15cm depth were used to determine "mineralization potential" over a 26 week period by a modification of the leaching method of Stanford & Smith (1972). Duplicate 25 g soil subsamples were added to glass funnel tubes (3.0 x 8.0 cm) with a pad of glass wool in place to retain the soil. The soil within the funnels was leached immediately with 100 ml of 0.01 M $CaCl_2$ and allowed to drain by gravity. The leachate volume was measured and aliquots used to determine total inorganic nitrogen by steam distillation after reduction with Devarda's alloy (Bremner, 1965).

The leaching funnels with soil were transferred to an environmental control room programmed to provide temperatures varied diurnally to be equivalent to the mean maximum and minimum temperatures at University Park, Pa. during July. This temperature program was changed monthly during the next 26 weeks to correspond to mean maximum and minimum temperatures during August, September, October, November, and December. At regular intervals, the soils were leached with 0.01M $CaCl_2$ and total mineralized N determined as described previously.

Some difficulty was experienced in obtaining adequate rates of leaching by the Stanford and Smith method, so this approach was discontinued midway through the incubation period with soil samples collected in October, 1974. A simple static incubation procedure as described below was employed in all subsequent mineralization studies.

Replicate 50 g soil samples with and without supplemental additions of 100 µg NH_4^+-N/g soil as $(NH_4)_2SO_4$ were added to 250 ml Erlenmeyer flasks, closed with Parafilm and incubated for varying time periods. For analyses of mineralized nitrogen, duplicate flasks were extracted with 200 ml of 2N KCl and the extracts used to determine NO_3^- and NH_4^+ by steam distillation (Bremmer, 1965). Generally, the incubation period was 1 month at temperatures equivalent to those at the time the soils were collected. As before, diurnal temperature programming was employed.

Denitrification Studies

The "denitrification potential" of soil samples from various profile depths, and from soil samples amended with glucose or plant residue was determined. The "denitrification potential" as defined for this study is the amount of added NO_3^--N denitrified within a specified time period when the soils are incubated under anaerobic conditions at constant temperature (usually 25°C). A 5 or 7 day period was employed.

To measure denitrification potentials, 25 g soil samples were placed in 115 ml screw-capped plastic bottles. Twenty-five milliliters of water containing 100 µg N/g soil as $NaNO_3$ was added to each bottle to create anaerobic conditions and supply NO_3^-. Controls received water without NO_3^-. The soil and water were mixed, the bottles closed and incubated for various time periods at 26 ± 1°C. Generally, triplicate soil samples were removed at 5 or 7 days for the extraction and measurement of residual nitrate or at 0,1,3 & 7 days when the time course of denitrification was being followed. The soil samples were extracted with 100 ml of water and NO_3^--N

determined in aliquots by steam distillation after reduction with Devarda's alloy (Bremner, 1965).

In certain studies, glucose was added to determine if the lack of a readily available source of carbon was limiting denitrification. In these studies the 25 g soil samples were amended with 25 ml of an aqueous solution containing 100 µg C/g soil as glucose and 100 µg N/g soil as $NaNO_3$. The anaerobic incubation and analytical procedures for nitrate determination were the same as those described above.

The effect of C/N ratios upon denitrification was also determined. Soil samples (25g) were treated with 25 ml water containing sufficient NO_3^--N to provide 100 µg N/g soil while the concentration of glucose carbon were varied to obtain different C/N ratios between 0.1 to 4.0. The soil samples were incubated for 7 days under anaerobic conditions and then extracted with water as described above. These extracts were analyzed for residual NO_3^--N.

Denitrification Studies After Residue Additions

Experiments were designed to evaluate the ability of plant residues at different stages of decomposition to serve as a carbon source for denitrification.

In the first experiment, residues were collected from the wastewater treated Reed Canarygrass, Old Field, and Hardwood Forest sites in the Autumn of 1974. The residues were dried at 65° C and ground with a stainless steel Wiley Mill to pass a 100 mesh screen. Replicate 50 g soil sub-samples from each site were transferred to 250 ml erlenmeyer flasks and amended with 0.5 g of plant residue from the same site. The flasks were adjusted to 1/3 atm. moisture %, covered with Parafilm, and the soils incubated for 1, 2, 3, 4, 6, 7, and 9 weeks at room temperature (26 ± 1°C). At the end of each designated incubation period, 50 ml water containing 100 µg N/g soil as $NaNO_3$, was added to duplicate samples of each treatment. The flasks were stoppered and incubated for 7 days under anaerobic conditions. Residual NO_3^- was determined as before to evaluate the "denitrification potential" of the amended soils.

The second experiment was a soil column study designed to provide more realistic conditions for residue decomposition and allow simultaneous measurement of evolved CO_2 during the experimental period.

Residues from wastewater treated Reed Canarygrass, Old Field and Hardwood Forest sites were collected in early November 1975. At the time of collection the quantity of surface plant residue found at each location was estimated. A total of 685 g/m^2 was found in the Reed Canarygrass area, 660 g/m^2 in the Old Field and 626 g/m^2 of leaves in the Hardwood Forest. If we assume complete soil incorporation of these residues, these additions would approximate a 0.3% amendment in the surface 15 cm of soil. The residues were dried at 65°C. Prior to soil incorporation all three residues were cut into 1.5-2.5 cm segments. This treatment was considered to more closely mimic the natural process of residue comminution and soil incorporation by the soil fauna, than that afforded by the previous use of finely ground residues.

Three hundred gram soil (oven dry weight) sub-samples from 7.5-15 cm depth of each vegetation area were transferred to 8.0 x 20 cm high impact styrene pipe. Then, 300 g soil sub-samples from the 0-7.5 cm depth were amended with 0.6% residue specific for each soil and transferred to the columns. Eight replicated soil columns and eight unamended control soil columns were adjusted to the 1/3 atm. moisture %, and an equal number were saturated with water. All of the soil columns were connected to a CO_2 scrubber and individual NaOH bubble towers for the measurement of evolved CO_2 during residue decomposition. The entire system and procedures have been described previously (Miller, 1974).

The temperature of the soil columns during incubation was varied diurnally and monthly to correspond to the mean maximum and minimum temperatures for April, May, and June in University Pk. Pa.

Measurements of the "denitrification potential" (5 day) for the amended and control soils were made initially, and at 1 week, 2 weeks, 1 month, 2 months, and 3 months. Duplicate soil columns were sacrificed at each time period, the soil removed mixed and 25 g sub-samples used for "denitrification potential" measurements as described previously.

Soil Available Carbon

A number of different procedures were used to estimate the availability of soil carbon for biological denitrification from different vegetation systems and depths in the soil profile.

Soluble organic carbon and carbohydrate equivalent carbon were determined following the procedures of Stanford et al. (1975). Five gram soil sub-samples were placed in 100 ml screw-capped plastic bottles with 25 ml of 0.01 M $CaCl_2$. These suspensions were heated at 100°C in an autoclave for 30 minutes and allowed to cool. Water lost during heating was replenished and the suspensions filtered. Two milliliter aliquots were analyzed for soluble carbon by the dichromate method (Graham, 1948) and for carbohydrate carbon by the anthrone method based on a glucose standard curve (Loewus, 1952).

Water soluble carbon and mineralizeable carbon were estimated following modifications of the procedures of Burfurd and Bremner (1975). Water soluble carbon was determined by extracting 10g soil sub-samples with 20 ml of distilled H_2O in a 50 ml centrifuge tube for 15 minutes with constant shaking. The suspensions were centrifuged for 60 minutes at 13800 x g using an International Model PR-2 Centrifuge, and the supernatants filtered through 47 mm dia. 0.2 µm Millipore Filters. Organic carbon in the filtered extracts was determined by the dichromate method of Graham (1948).

Mineralizable carbon was determined on duplicate 50 g soil samples at field moisture content incubated in 6 x 6 cm screw capped jars for 7 days. Evolved CO_2 was collected in 0.2 N NaOH, precipitated with $BaCl_2$, and determined by titration with standard HCl using phenolphthalien as the indicator.

The "light fraction" of the soil organic matter from some of the experimental soil samples was determined by the method of Ford et al (1969). Five grams soil,

previously dried for 16 hours at 65° C, was mixed with 100 ml of Fumazone* and dispersed for 5 minutes with a Branson Model W-185C Ultrasonic Probe in an ice bath. After dispersion the sample was allowed to settle for 1 hour then centrifuged. The supernatant with the "light fraction" floating on the surface was decanted and filtered through a 0.45 μm Millipore filter. The separated "light fraction" organic matter on the filter was washed with acetone, then oven dried at 65° C and weighed.

Studies With Undisturbed Soil Cores (Reed Canarygrass Site)

Undisturbed soil cores (15 x 10 cm) were obtained from the Wastewater treated and Control Reed Canarygrass site and used to evaluate nitrogen renovation efficiency during irrigation with a simulated effluent.

Cylinder cut from iron pipe (20 x 10 cm with a 2 mm wall thickness) were machine beveled to provide a cutting edge for soil penetration. The cylinders were painted inside and out with a rust inhibiting paint, dried at 100° C and thoroughly washed with H_2O before use. A special adapter was fabricated to attach the cylinders to the Kelly bar of a truck mounted Giddings hydraulic power probe used to insert the cylinders into the soil. Twenty undisturbed soil cores were removed from the treated and 10 cores from the control Reed Canarygrass sites in April 1975. The cores were wrapped in polyethylene and returned to the laboratory at Ohio State University. The soil cores were fitted with sheet metal bottom caps containing a center mounted 5 x 1 cm copper drainage tube. The caps had previously been painted with a rust inhibiting paint. A pad of glass wool was inserted between the bottom soil surface and the cap. The completely assembled soil cores were set up vertically within a Sherer environmentally controlled growth chamber. The integrity of all of the cores was evaluated by leaching them with distilled H_2O and observing the rate of infiltration. A few cores were discarded because of unusually rapid infiltration indicating a crack or an earthworm burrow.

A total of 18 cores from the wastewater treated site and 9 cores from the control site were distributed in a randomized block design (3 blocks x 3 reps/block) under the light bank. All of the columns were watered with distilled H_2O and allowed to aclimate for 17 days before the beginning of the wastewater treatment phase. The environmental parameters were as follows:

Temperature range; 26.5°C day temperatures, 15°C night temperatures
(Equivalent to July temperatures at University Park, Pa)

Light; a 12 hour day and 12 hour night
(Light intensity ~ 3000 ft. candles at plant height)

Prior to the start of the treatment phase the established vegetation was cut back to about 2.5 cm in length. Treatment variables were a simulated effluent (See Appendix B) at rates equivalent to 0, 2.5 and 5 cm/week at an application rate of 0.25 cm/hr. An additional application of 2.5 cm/week distilled water was made

* A Dow Chemical Co. trade name for 1,2-dibromo-3-chloropropane often called (Nemagon) (density of 2.06 g/cm^3) was diluted to a density of 2.0g/cm^3 with hexane before use.

weekly to simulate rainfall events and was applied 2 days after the effluent application. Drainage water was collected after each irrigation or rainfall event. The volume of leachate was measured and analyzed for pH, NH_4^+ N and NO_3^--N .

Nitrogen analysis was by steam distillation following the procedures of Bremner (1965).

Vegetative growth was harvested every 4 weeks, dried at 70°C, weighed and ground in a Wiley Mill. Nitrogen analysis of the plant material was by micro-kjeldahl procedures using a selenium catalyst.

Reed Canarygrass Root Mass Measurements

Seasonal changes in root mass were estimated on the wastewater treated Reed Canarygrass site. Three undisturbed 5 x 30 cm soil cores were removed with a Giddings Power Probe from each of 12 points along a transect parallel to and about equi-distant between irrigation lines 8 and 9. The sampling points were spaced about 21 m apart. The cores were removed from the sampling tube, placed in plastic bags and returned to the laboratory.

The 3 soil cores from each sampling point were combined in a single container and soaked in H_2O for 24-48 hours. The roots were separated from the soil by passing the soaked soil cores over a 50 mesh screen with repeated washings. The washed roots were collected , dried in an oven at 70° C and weighed. The dried root materials was ground in a Wiley Mill and analyzed for organic N by the micro-kjeldahl procedure and for organic C by dry combustion.

RESULTS AND DISCUSSION

Nitrogen Mineralization and Nitrification

One parameter necessary for the calculation of soil nitrogen balances in soils receiving wastewater is nitrogen mineralized from soil organic nitrogen. Assuming that each soil with associated vegetation has a certain finite renovative capability for nitrogen, then, the larger the quantity of soil derived nitrogen the lower the amount of wastewater nitrogen which can be added before excess nitrogen occurs in the soil profile. Generally, in soils of humid-temperate regions a value of 3-5% mineralization of the soil organic nitrogen can be expected annually, or more correctly, during the months when the soils are warm enough for microbial activity.

This study used two different approaches to provide a value for soil organic nitrogen mineralization from the different soil-vegetation systems of the Penn State Wastewater Management Area. The first method involved a prolonged 26 week incubation-leaching experiment following the procedures of Stanford and Smith (1972). Data for the surface 0-7.5 cm samples are graphed in Fig. 1 and for the 7.5-15 cm samples in Fig. 2 as N mineralized vs. $t^{\frac{1}{2}}$. (Stanford and Smith (1972) have previously shown that a plot of N mineralized vs $t^{\frac{1}{2}}$ is linear for most soils incubated at constant temperatures. In this study, N mineralization from the surface 0-7.5 cm soil zone of both wastewater treated and control sites was essentially linear for the 26 week incubation period (Fig. 1). Deviation from linearity occur primarily in the last two data points. Incubation temperatures during this period were equivalent to November and December and below optimum for microbial activity. N mineralization from the 7.5-15 cm soil zone was linear up to 8 weeks with no mineralization thereafter (Fig. 2). This lack of mineralization undoubtedly reflects the depletion of mineralizable organic N compound early in the incubation period. The marked decrease in organic N with depth (Table 2) certainly supports this concept.

Nitrogen mineralization in the wastewater treated soils was higher than control soils for all but the Old Field site. This is shown by a comparison of the slopes of the lines of Fig. 1 as recorded in Table 4. The reason for the reduced mineralization in the wastewater treated Old Field soils is not known, but certainly does not reflect the higher organic N content of this soil compared to the control soil. This aspect requires further investigation in light of the observation that the Old Field system does such an excellent job of nitrogen renovation without annual removal of vegetation (Kardos and Sopper, 1973). Immobilization of nitrogen in humified weedy vegetation with slow mineralization could certainly be a contributing factor to this successful renovation.

The quantity of nitrogen mineralized from the 0-7.5 cm zone of the Morrison sandy loam from the Gameland area was of particular interest and concern. (Fig. 1 and Table 4). The absolute quantity of mineralized N from the wastewater treated area was about 5 to 9 times greater than any of the other treated areas. Even the nitrogen mineralized from the control areas was 1.5 to 2.0 times greater than from the other soil-vegetation systems. This very high N mineralization rate may explain the high NO_3^--N found in field suction lysimeters at the 120 cm depth in this area (See Fig. 7-4, Kardos and Sopper, 1973). There can be little doubt that the mineralization of 448 kgN/ha (Table 4) in addition to that supplied by

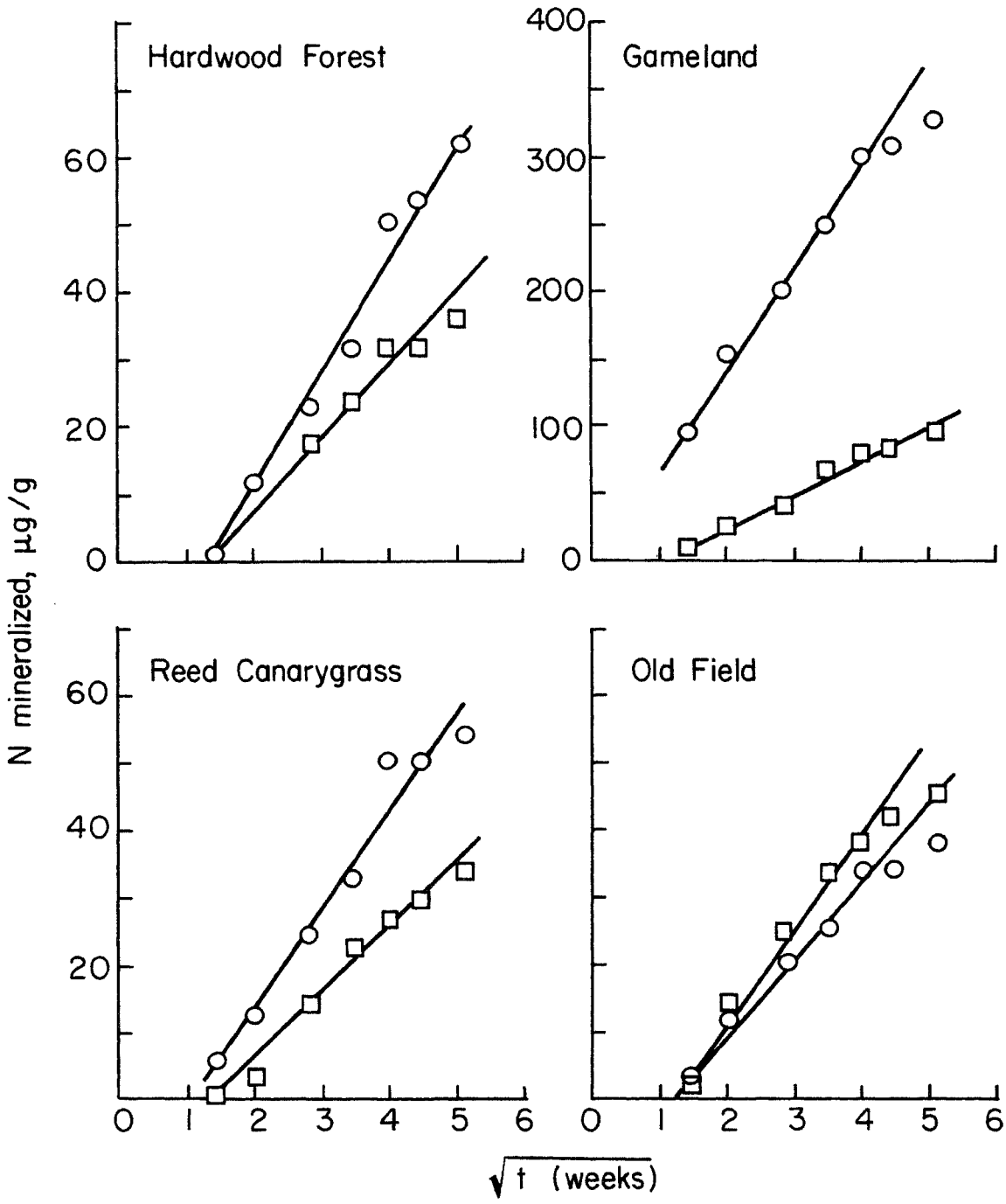

Figure 1. Cumulative N Mineralized vs the Square Root of Incubation Time for Soil Samples from the 0-7.5 cm Soil Depth.

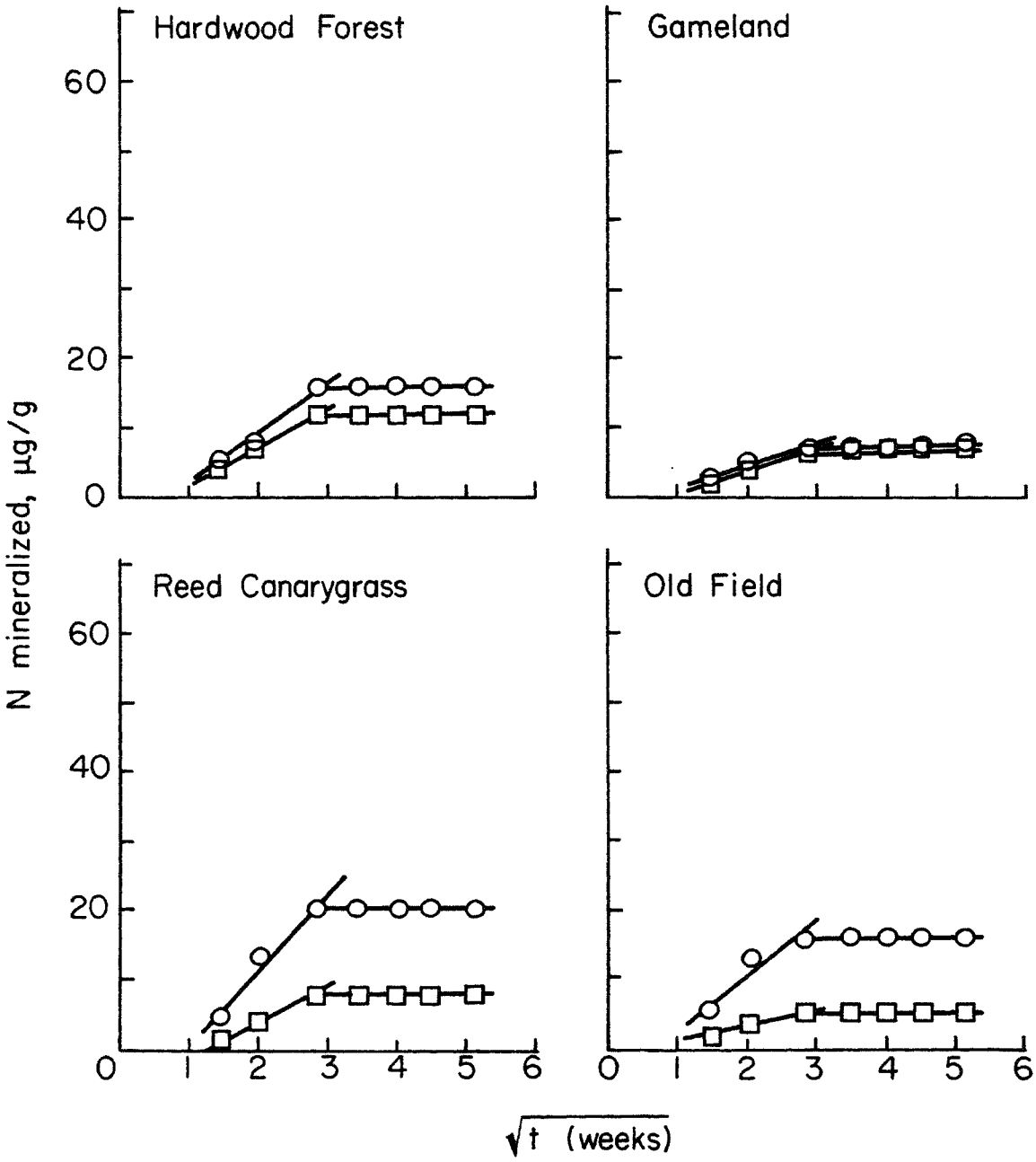

Figure 2. Cumulative N Mineralized vs the Square Root of Incubation Time for Soil Samples from the 7.5-15 cm Soil Depth.

Table 4. Estimated N Mineralized from Wastewater Treated and Control Soils. (Penn State University Wastewater Management Area.)

Soil Vegetation	Soil Depth	Treated Area			Control Area		
		ug N/g*/ week 1/2	N Mineralized ** (8 months)	% of organic N	ug N/g*/ week 1/2	N Mineralized ** (8 months)	% of organic N
	cm		Kg/ha			Kg/ha	
Reed	0-7.5	19.0	113.7	4.0	12	71.8	2.8
Canarygrass	7.5-15	11.3	23.9	1.6	5	10.4	0.7
			Σ =137.6			Σ =82.2	
Hardwood	0-7.5	14.5	116.6	3.5	10.3	61.3	3.3
Forest	7.5-15	7.8	16.4	1.5	6.0	12.7	1.0
			Σ =133.0			Σ =74.0	
Old	0-7.5	11.0	65.7	2.0	12.3	73.2	4.6
Field	7.5-15	8.2	17.1	1.6	2.1	4.5	0.6
			Σ =82.8			Σ =77.7	
Gameland	0-7.5	73.8	441.1	8.3	27.5	164.5	7.9
	7.5-15	3.2	6.7	1.4	3.2	6.7	1.4
			Σ =447.8			Σ =171.2	

* Values represent the slope of the lines, Fig. 1 & 2. Only the first 3 data points are used for the Fig. 2 data.

** Calculated from the mineralization data of Fig. 1 & 2 assuming linearity of mineralization rate for 32 weeks in the 0-7.5 cm samples but only 8 weeks in the 7.5-15.0 cm samples.

the wastewater would contribute to excess nitrogen capable of being leached downward. It appears that this increased mineralization rate is a reflection of greater accessability of the organic N in the coarse textured soil to microbial catabolic activity. Factors such as organic N occlusion within aggregates or peds, or enzyme or organic N immobilization on clay mineral surfaces limits decomposition in fine textured soils. Clapp (1972) has also related the quantity of mineralized N in soils to the percentage of soil N contained in the light fraction organic matter. Analyses for light fraction material was done in this study but the quantity or organic carbon and nitrogen recovered from all the experimental soils and treatments was so low that it was considered insignificant with respect to its contribution to mineralizable nitrogen.

The data in Fig. 1 and 2 were used to estimate the total quantity of nitrogen which would be mineralized during a proposed 8 month wastewater application period (common for the Penn State Wastewater Management Area). This calculation was based on the assumption that the mineralization rate during April, May , and June would have been similar to that determined for July to November (again see Fig. 1 & 2). These estimates were expressed as kg N/ha of mineral N and mineral N as % of soil organic N (Table 4). A number of inferences can be made from these data. For example, most of the mineralized nitrogen is derived from the organic nitrogen of the surface 7.5 cm of soil. The organic nitrogen of the 7.5-15.0 cm zone is much more resistant to mineralization and behaved remarkably similar regardless of the surface vegetation or wastewater treatment. It is also the surface 7.5 cm soil zone which has been most modified by continual wastewater treatment resulting in a pool of potentially mineralizable mineral nitrogen. Perhaps a measure of potentially mineralizable N could best be estimated by considering only this surface soil zone.

Nitrogen mineralization rates were also determined by measuring the quantity of nitrogen mineralized from soil samples (0-15 cm depth) incubated for 4 weeks at the temperature for the month in which the soil sample was collected. Data from April, August and November are plotted in Fig. 3. These data show that the mineralization rates of the wastewater treated soils follow the same order as found by the long term incubation leaching study (Fig. 1) i.e. Gameland > Reed Canarygrass = Hardwood Forest > Old Field. The control soils, however, behaved differently in the Reed Canarygrass and Hardwood Forest soils with a mineralization rate near that of the wastewater treated soils.

The average monthly mineralization rate for each site (Fig. 3 data) for the 4 months incubation period was multiplied by 8 to provide an additional estimate of the total amount of N mineralized from organic N from April-November. These data were expressed as % of the soil organic N mineralized in the surface 15 cm of soil and compared with the previous calculation found in Table 4. The comparison of data is shown in Table 5. The agreement between the two sets of data were very good for the wastewater treated soils but more erratic for the control soils. This erratic data was a reflection of the high mineralization in the control sites of the Reed Canarygrass and Hardwood Forest area found by this method. Note, however, that the general assumption that 3% of the soil organic N is mineralized annually in the humid temperate soils would hold for the three wastewater sites on the Hublersburg soil. The possibility that organic N from sites on coarse textured soils mineralize more rapidly should be investigated, since it would make these soils less useful for renovation than previously assumed.

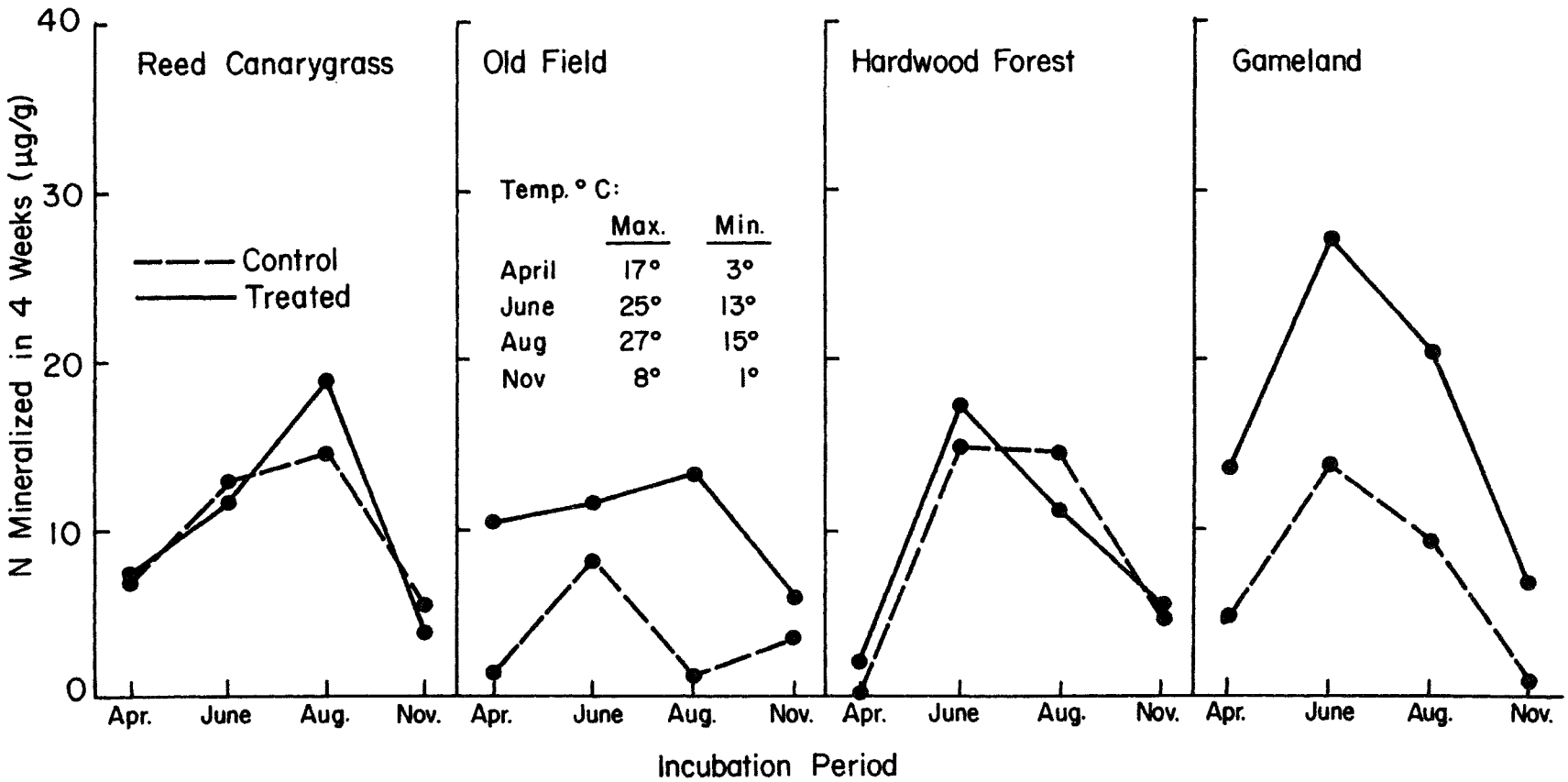

Figure 3. Nitrogen Mineralized in Soils From Four Wastewater Management Sites During a 4 Week Incubation at the Temeratures Indicated (0-15 cm depth).

Table 5. Comparison of Percent Soil Organic Nitrogen Mineralized by Two Methods * (0-15 cm depth)

Soil Vegetation	Treated Area		Control Area	
	1	2	1	2
	%		%	
Reed Canarygrass	3.2	3.2	2.0	4.0
Hardwood Forest	3.0	3.4	2.4	3.7
Old Field	1.9	2.7	3.3	2.1
Gameland	7.8	8.5	6.7	4.9

* Method 1- Used data from Fig. 1 & 2 and Table 4 and Organic N %, Table 2.
Method 2- Used mineralization data, Fig. 3, and Organic N%, Table 3.

The previous data considered only total mineralized N without attempting to partition the mineral N between NH_4^+ and NO_3^-. This portion of the study examined the relative rate of nitrification in wastewater treated and control soils from the different soil-vegetation systems. Nitrification is a process of considerable importance in soil wastewater renovation systems since rapid nitrification means a greater potential for leaching of NO_3^- as well as for loss of nitrogen through denitrification. In addition, the literature contains some reports that nitrification is frequently inhibited in forest soils and grassland soils (Clark and Paul, 1971). Since two of the experimental sites were forested and one (the Reed Canarygrass area) is a perennial grass, this present evaluation seemed particularly significant.

Data for changes in total, NH_4^+ and NO_3^- during a 4 week incubation period at the temperatures indicated are given in Figures 4, 5, 6, and 7. Data for nitrification in soils amended with 100 ug/g soil of NH_4^+ -N are included to indicate the rate of nitrification with large additions of NH_4^+-N. It can be seen that NO_3^- is the dominant form of inorganic N in all the unamended wastewater treated soils throughout the incubation period. Ammonium when present was less than 5 ug/g. Likewise, added NH_4^+ nitrogen is rapidly nitrified in all soils and at all temperatures, even November temperatures. These data certainly indicate that forest litter, or Reed Canarygrass does not inhibit nitrifying bacteria. Nitrification did not occur, however, in the control soils of the Old Field or Gameland sites and was slow in the Hardwood forest control area (Data not shown). The lack of nitrification in these locations can be readily explained by the low pH of these control sites (Table 3). It is well recognized that the rate of nitrification falls off markedly in soils with a pH below 6.0 and becomes negligible below pH 5.0 (Alexander, 1977).

Population estimates of nitrifying bacteria were made by an MPN method on soil samples from two depths collected in August and November. These data are presented in Table 6. In general these population estimates add nothing to the previous discussion of nitrification in the soils of the wastewater treatment areas. A population of 10^3 or 10^4/gram of soil are considered normal for soils of moderate organic matter content which have not received massive additions of NH_4^+ -N or manures. No explanation can be given for the lower population of nitrifying bacteria in August than November. As expected the population was generally (but not always) lower in soils from the 7.5-15 cm depth than from the surface 0-7.5 cm zone. There was also a general tendency for the nitrifying bacteria to be lower in the control soils than the wastewater treated soils. Note, however, that nitrifying bacteria were present in the control soils of the Hardwood Forest, Old Field, and Gameland soils where nitrification was found to be absent or occur at a very slow rate.

We have concluded that population estimates of nitrifying bacteria using the MPN method is not a useful parameter for evaluating the potential of soils for nitrification. A similar conclusion has recently been expressed by E. L. Schmidt (University of Minnesota, personal communication) after an extensive study of nitrifying bacteria in soil.

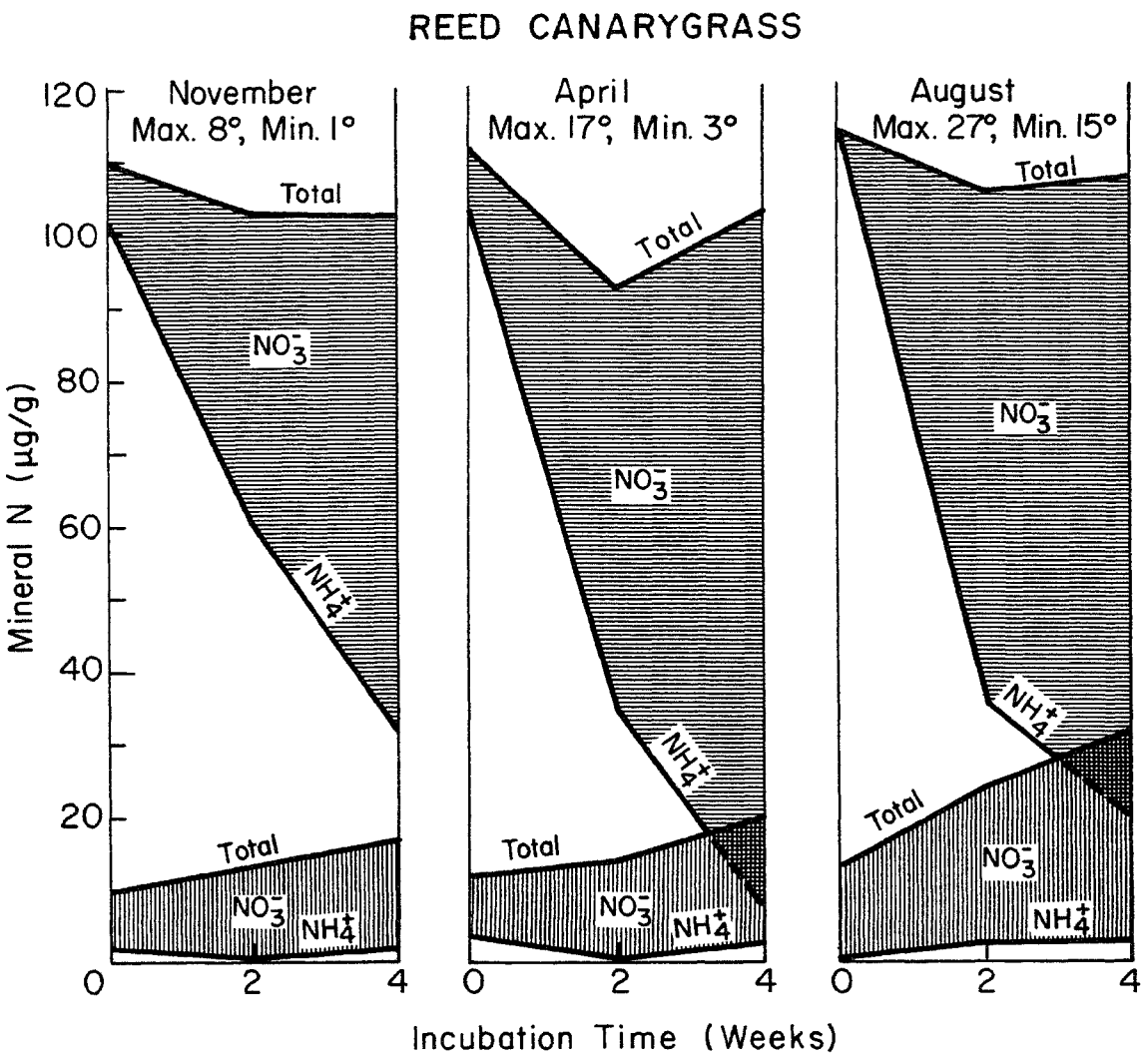

Figure 4. Relative Rate of Nitrification in Wastewater Treated Reed Canarygrass Soil at Different Seasonal Temperatures (0-15 cm Soil Depth).

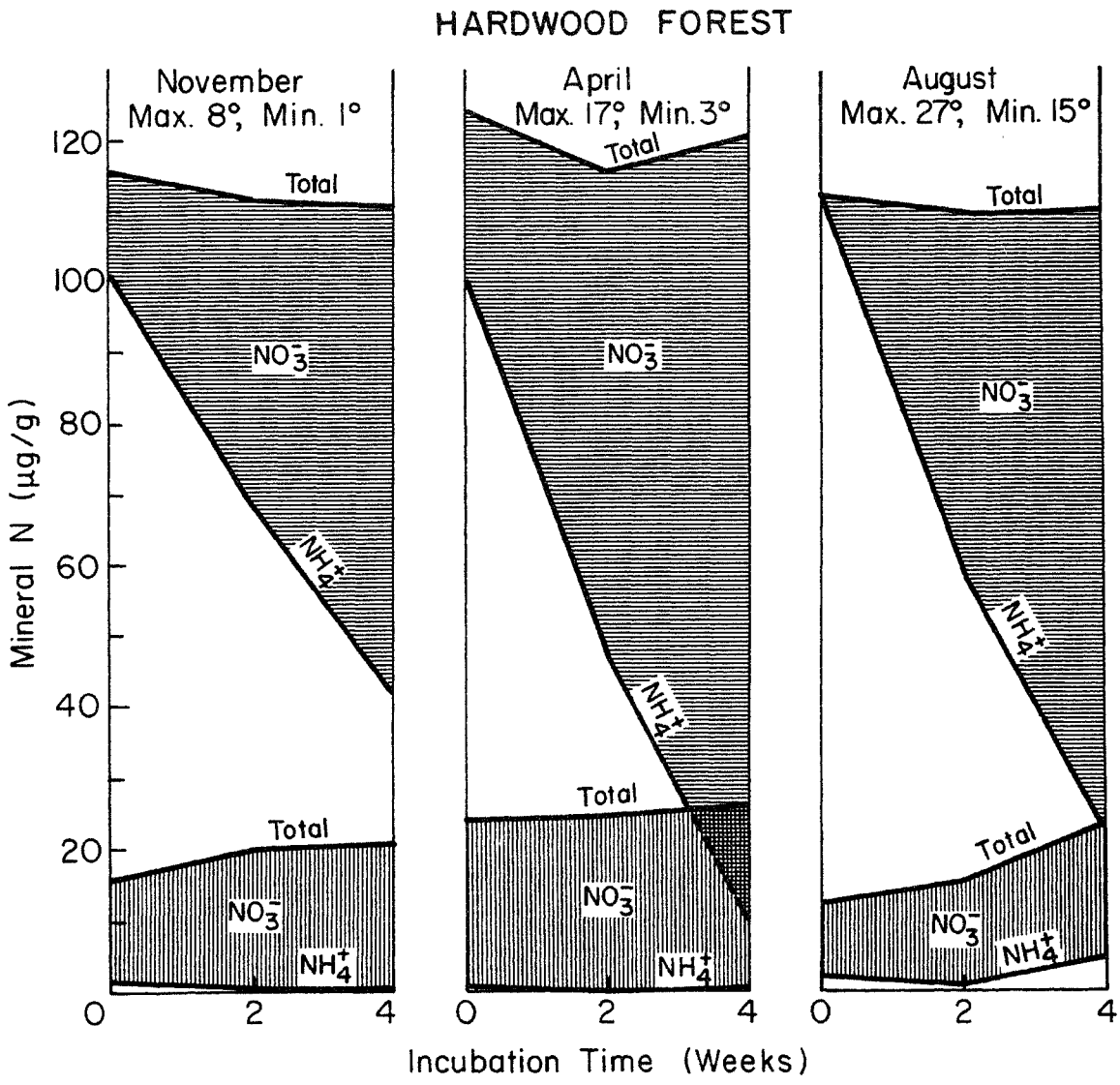

Figure 5. Relative Rate of Nitrification in Wastewater Treated Hardwood Forest Soil at Different Seasonal Temperatures (0-15 cm Soil Depth).

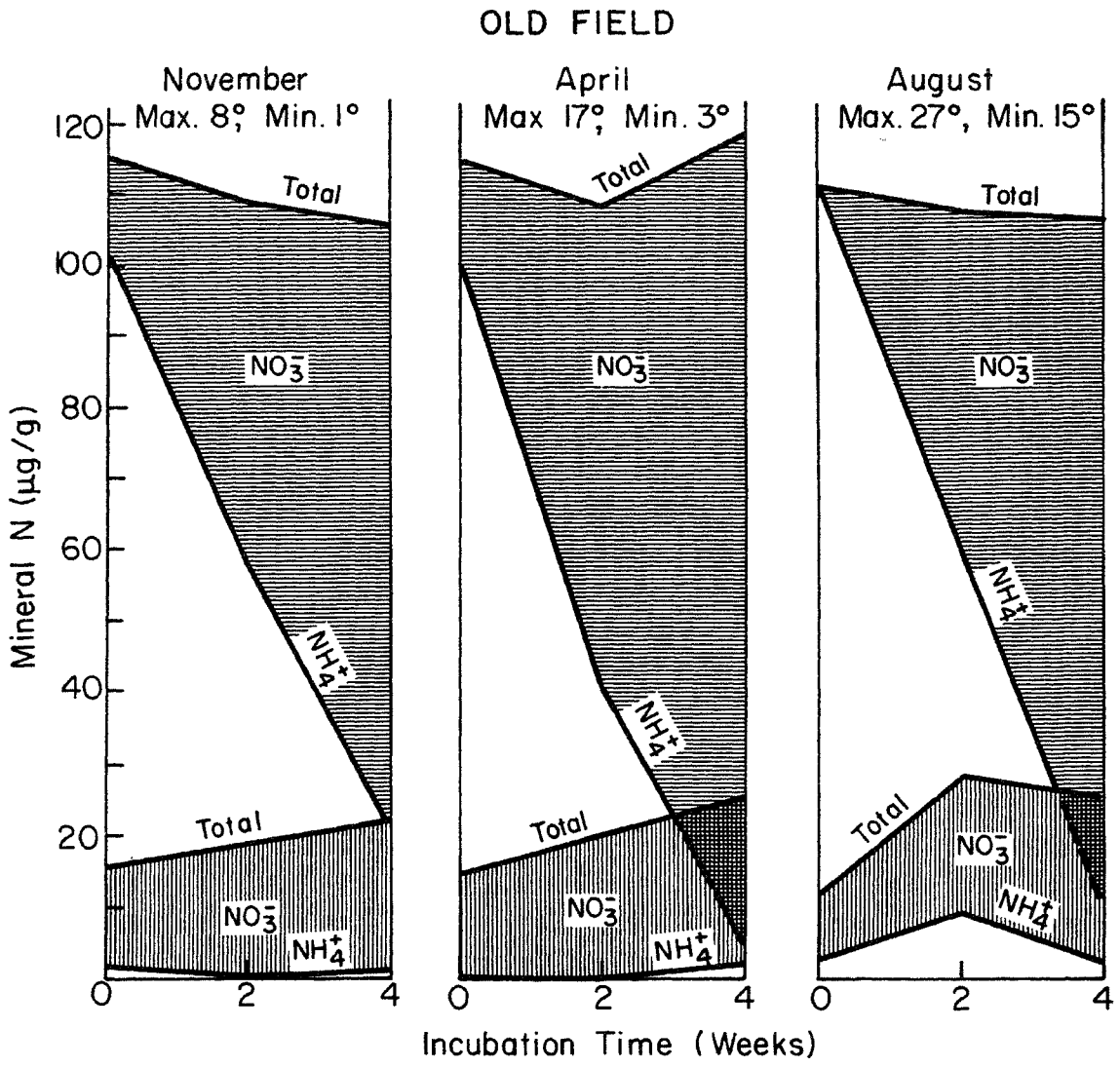

Figure 6. Relative Rate of Nitrification in Wastewater Treated Old Field Soil at Different Seasonal Temperatures (0-15 cm Soil Depth).

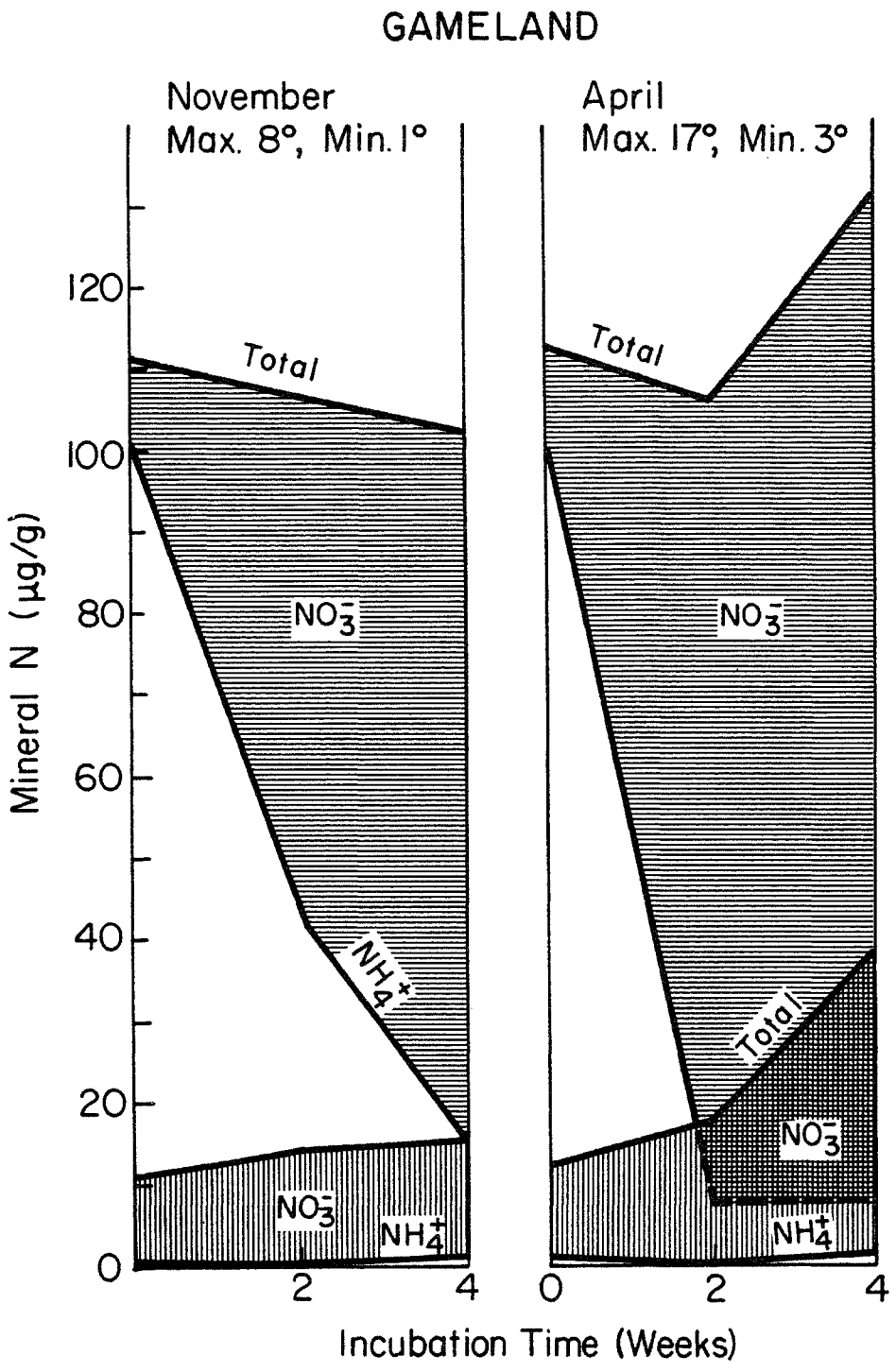

Figure 7. Relative Rate of Nitrification in Wastewater Treated Gameland Soil at Different Seasonal Temperatures.

Table 6. Estimated Number of Nitrifying Bacteria in Wastewater Treated and Control Soils

Wastewater Site		Sampling Time: August	Sampling Time: November
		No./g dry soil	
Reed Canarygrass (Treated)	0-7.5 cm	2.1×10^3	3.8×10^4
	7.5-15 cm	1.7 "	4.7 "
(Control)	0-7.5 cm	2.6 "	7.3 "
	7.5-15 cm	1.4 "	-
Hardwood Forest (Treated)	0-7.5 cm	1.4×10^3	5.1×10^4
	7.5-15 cm	1.6 "	4.5 "
(Control)	0-7.5 cm	0.07 "	1.1 "
	7.5-15 cm	2.5 "	1.3 "
Old Field (Treated)	0-7.5 cm	14.2×10^3	3.1×10^4
	7.5-15 cm	4.6 "	5.8 "
(Control)	0-7.5 cm	10.4 "	3.3 "
	7.5-15 cm	3.7 "	-
Gameland (Treated)	0-7.5 cm	5.6×10^3	5.2×10^4
	7.5-15 cm	0.3 "	2.6 "
(Control)	0-7.5 cm	4.4 "	2.7 "
	7.5-15 cm	0.5 "	4.2 "

Factors Affecting Denitrification

Nitrogen uptake by plants and biological denitrification are the most important processes for the renovation of nitrogen from wastewater by land treatment systems. Biological denitrification is thought to be the major mechanism for removal of nitrogen in excess of that utilized by plants and particularly for NO_3^- leached beyond the root zone (Lance, 1972, Miller, 1973). Denitrification losses have generally been assumed to be higher in soils receiving wastewater than in normal agricultural soils because the higher hydraulic loadings should keep the soil more water saturated and provide more anaerobic zones and microsites within the soil profile.

This portion of the study was designed specifically to evaluate the potential for biological denitrification in soils of the Penn State Wastewater Management sites. Particular emphasis was given to an evaluation of the presence of sufficient available carbon to support biological denitrification in the surface soil zones as well as sub-soils below the root zone. These sub-soils are very low in carbon and might not support denitrification unless soluble carbon would leach downward from the surface soil horizons. This seemed highly unlikely because of the fine texture of the Hublersburg soil which would probably adsorb any mobile organic matter in the surface 15 cm of the soil profile. A lack of denitrification in lower soil zones means that NO_3^- leaching beyond the root zone would remain stable and eventually intercept and contaminate the underlying groundwater.

The "denitrification potential" of soils from different depths and under different vegetation at the Penn State Wastewater Management site was measured on soil samples collected in July and October, 1974. The data from these experiments are shown in Table 7. Denitrification in all soils was low and did not show any consistent relationship with wastewater treatment, soil depth, type of vegetation or sampling time.

Low denitrifying activity in the 15-30 cm and 30-60 cm depths was expected because of the low organic carbon content of the soil profile at these depths (see Table 2). The denitrifying activity in the surface soil samples was however, far below that expected. Poor denitrification in these surface soil subsamples could be due to one or more of the following reasons:

1. An unusually low population of denitrifying bacteria.

2. Absence of easily decomposable organic carbon compounds for the heterotrophic denitrifying bacteria.

3. Accumulation of substances toxic to the denitrifying bacteria.

These alternate hypotheses were evaluated and the data from these experiments are presented and discussed in the following paragraphs.

The population of denitrifying bacteria in wastewater treated and control soils from all four sites, at three different seasonal sampling times, and at four different depths was estimated using Most Probable Number (MPN) techniques. These data are shown in Fig 8 , 9 , 10 and 11 . The population of denitrifying bacteria decreased from the surface downward as expected. Numbers of denitrifying bacteria in the 0-7.5 and 7.5-15 cm depths, however, are greater than 10^6/g, a

Table 7. Denitrification Potentials of Wastewater Treated and Control Soils From Different Depths and Uncer Different Vegetation

Month	Depth	Reed Canarygrass		Hardwood Forest		Old Field		Gameland	
		Control	Treated	Control	Treated	Control	Treated	Control	Treated
	cm	% loss of added NO_3-N							
July 1974	0-7.5	12.8	0.0	0.0	8.4	0.0	0.0	0.0	20.3
	7.5-15	0.0	3.5	19.1	25.1	5.1	0.0	0.0	0.0
	15 -30	0.0	0.0	0.0	0.0	12.7	0.0	0.0	0.0
	30-60	0.0	0.0	0.0	0.0	13.9	0.0	0.0	0.0
October 1974	0-7.5	0.0	0.0	0.0	13.8	0.0	7.1	0.0	0.0
	7.5-15	0.0	0.0	0.0	0.0	0.0	3.1	0.0	0.0
	15-30	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	30-60	0.0	0.0	2.7	0.0	0.0	0.3	0.0	0.0

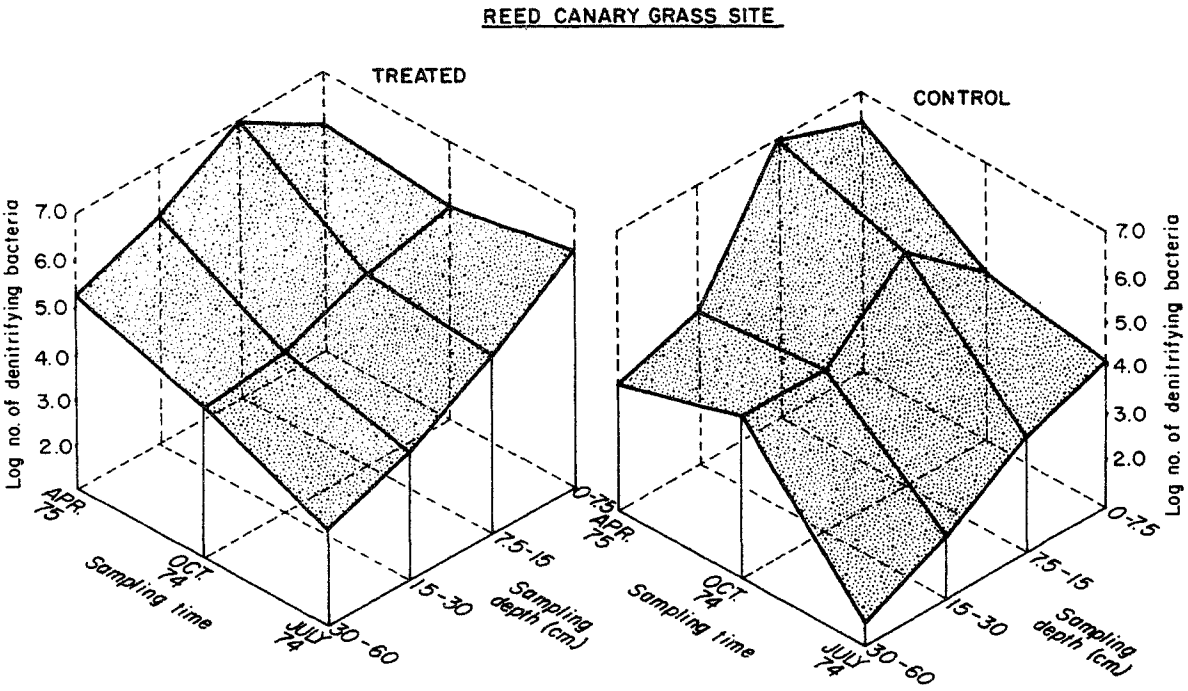

Figure 8. Population of Denitrifying Bacteria Found in Wastewater Treated and Control Soils with Depth and Sampling Time (Reed Canarygrass Site).

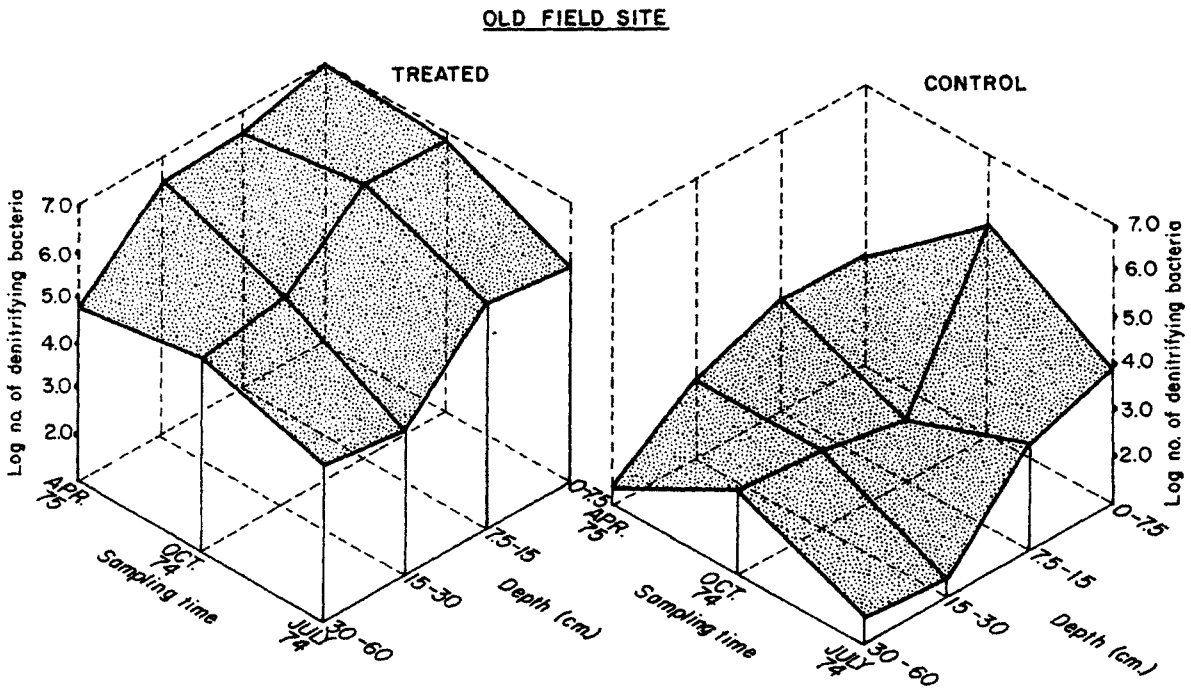

Figure 9. Population of Denitrifying Bacteria Found in Wastewater Treated and Control Soils with Depth and Sampling Time (Old Field Site).

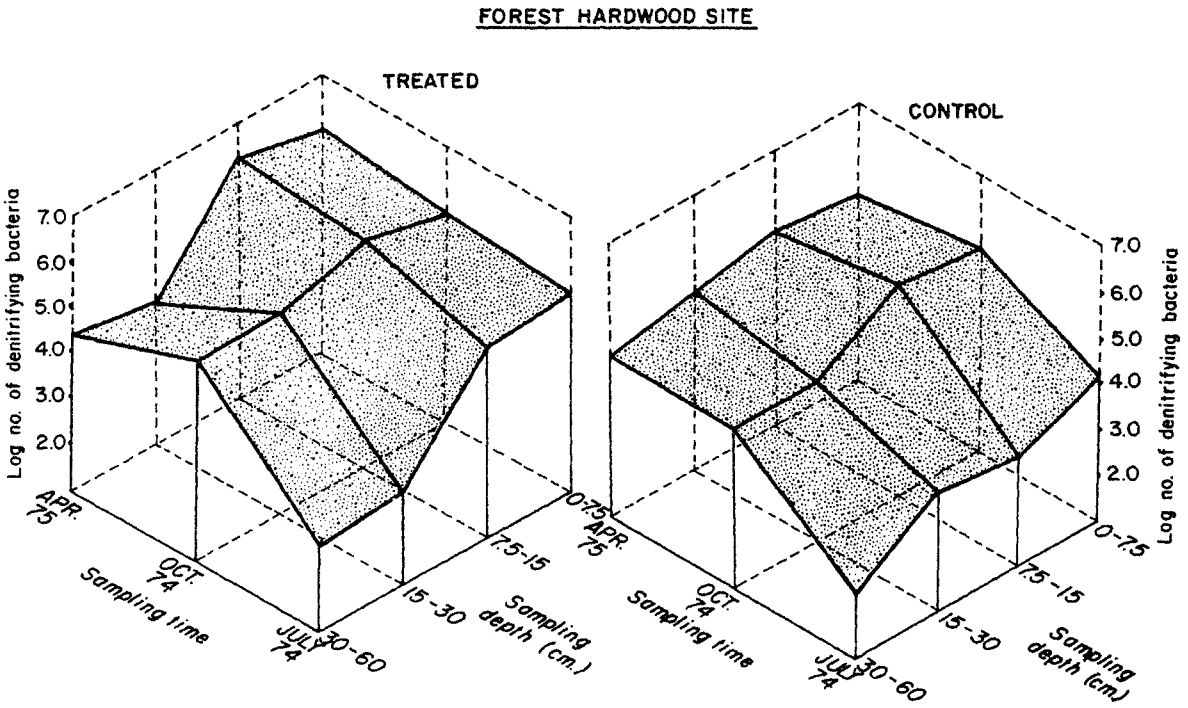

Figure 10. Population of Denitrifying Bacteria Found in Wastewater Treated and Control Soils with Depth and Sampling Time (Hardwood Forest Site).

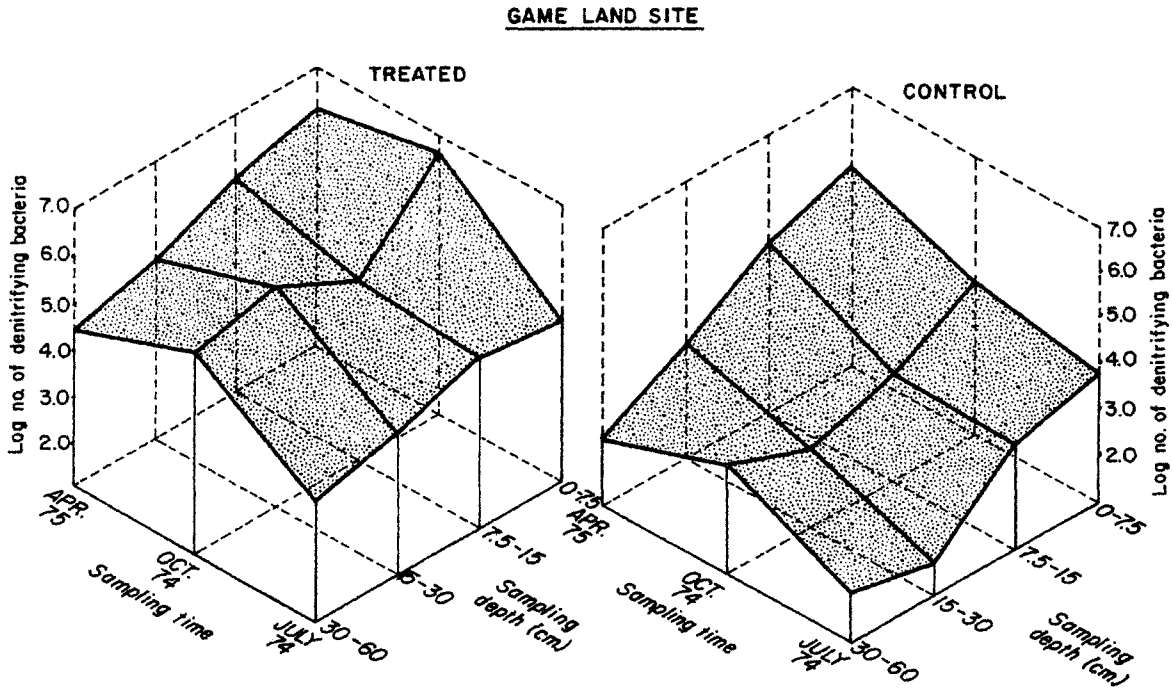

Figure 11. Population of Denitrifying Bacteria Found in Wastewater Treated and Control Soils with Depth and Sampling Time (Gameland Site).

Table 8. Soluble Organic Carbon and Glucose Equivalent Carbon From Different Depths of Wastewater Treated and Control Soils.

Soil-Vegetation System	Depth	Soluble Carbon		Glucose Eq. Carbon	
		July	Oct.	July	Oct.
	cm	ug/g		ug/g	
Reed Canary-grass					
	0-7.5	690	513	195	201
Treated	7.5-15	225	132	60	64
	15-30	89	32	21	28
	30-60	0	0	17	19
	0-7.5	534	322	175	137
Control	7.5-15	241	111	89	47
	15-30	97	0	32	19
	30-60	47	0	16	5
Old Field					
	0-7.5	715	888	208	188
Treated	7.5-15	142	196	52	50
	15-30	55	113	23	16
	30-60	0	231	17	12
	0-7.5	479	644	166	134
Control	7.5-15	144	67	46	43
	15-30	0	285	10	17
	30-60	0	211	20	8
Hardwood Forest					
	0-7.5	910	779	246	250
Treated	7.5-15	224	148	54	64
	15-30	108	26	25	24
	30-60	182	27	19	15
	0-7.5	749	454	217	143
Control	7.5-15	273	193	107	75
	15-30	128	152	54	51
	30-60	0	34	28	33
Gameland					
	0-7.5	1702	1166	424	360
Treated	7.5-15	306	221	76	35
	15-30	92	-	14	18
	30-60	0	153	18	11
	0-7.5	-	491	404	133
Control	7.5-15	178	221	52	52
	15-30	91	263	24	12
	30-60	0	313	27	183

Table 9. Quantity of Water Soluble Organic Matter and Carbon in Soils At Different Depths in Wastewater Treated and Control Soils (April Sampling)

Soil-Vegetation System	Soil Depth			
	0-7.5 cm	7.5-15 cm	15-30 cm	30-60 cm
	μg/g dry soil			
Reed Canary-grass				
Treated	128.8 (51.5)*	137.6 (55.0)	65.2 (26.1)	35.1 (14.0)
Control	50.4 (20.2)	45.6 (18.4)	18.8 (7.3)	10.2 (4.1)
Old Field				
Treated	73.8 (29.5)	97.0 (38.8)	44.2 (17.7)	15.3 (6.1)
Control	86.4 (34.6)	55.4 (22.2)	38.2 (15.3)	37.0 (14.8)
Hardwood Forest				
Treated	97.2 (38.9)	71.0 (28.4)	30.9 (12.4)	18.2 (7.3)
Control	50.9 (20.4)	37.6 (15.0)	0.0 (0.0)	0.0 (0.0)

* The values in parentheiss ( ) are estimated values for soluble carbon assuming that the extracted organic matter is similar to carbohydrate carbon ($\sim$ 40% C)

Table 10. Carbon Mineralized in 1 Week From Soil At Different Depths of Wastewater Treated and Control Soils (April Sampling)

Soil-Vegetation System	Soil Depth			
	0-7.5 cm	7.5-15 cm	15-30 cm	30-60 cm
	μg/g dry soil			
Reed Canary-grass				
Treated	96.0	61.0	26.4	41.1
Control	68.6	42.0	40.5	22.6
Old Field				
Treated	108.2	62.3	21.4	30.6
Control	95.3	29.2	27.0	20.5
Hardwood Forest				
Treated	87.6	53.3	51.2	24.0
Control	76.6	46.7	31.1	30.0

population considered normal for arable soils. All control sites had lower numbers of denitrifying bacteria than wastewater treated sites with the difference being particularly large in the Old Field soil. The population of denitrifying bacteria also increased from July to April which probably reflects increased available carbon in autumn and early spring from residue additions and release of organic matter by freezing and thawing. Overall, the population data for denitrifying bacteria will not explain the absence of denitrifying activity in the wastewater treated soil samples. Although the low population in sub-surface soils and control soils could decrease the denitrification activity, the population in the surface soil zone of wastewater treated soils is certainly adequate for rapid denitrification.

A series of experiments were designed to investigate whether a lack of available carbon was responsible for the reduced denitrification activity (low "denitrification potentials") in the surface 0-15 cm soil depths (Table 7). In the first experiment composite soil samples were amended with 1% glucose and "denitrification potential" measurements were made on soil samples incubated at 1/3 atm. moisture % (considered aerobic) and under saturated conditions (anaerobic). It was assumed that if available carbon was limiting, denitrification should increase when glucose is provided. Data for this experiment (Figs. 12, and 13) show that almost all added NO_3^- disappeared in 3 to 7 days in all soil samples from all sites incubated under saturated conditions. Even some decrease in NO_3^--N occurred under more aerobic conditions which might indicate that some anaerobic microsites occurred with intensified microbial activity in the glucose amended soils. In general, the wastewater treated soil samples supported a faster rate of denitrification than the control soil samples, and more nearly reflect the differences in population of denitrifying bacteria found between wastewater treated and control soils (Fig 8-11). These experimental results suggest that a lack of available carbon limits denitrification in soils of the Penn State Wastewater Management areas.

In a related study, an attempt was made to ascertain how much carbon (as glucose) would be necessary to maximize the loss of NO_3^- from the soil via biological denitrification. The data obtained are shown in Fig. 14 and show that a C/N ratio of at least 2 was necessary for maximum denitrification. This would mean that about 200 µg C/g soil would be necessary to completely denitrify the 100 µg/g of added NO_3^--N. Approximately 40-50 µg/g soil of glucose-C was necessary to initiate measurable denitrification. These data agree with the conclusions of Bowman and Focht (1974) but differ from empirical calculation of Burford and Bremner (1975) that 0.97 µg of available carbon is needed to produce 1 µg of $(N_2O + N_2)$-N.

During the past two years Burford and Bremner (1975) and Stanford et al (1975) have concluded that the magnitude of denitrification in soils under anaerobic conditions, is controlled by the supply of readily decomposable organic matter. Both groups have proposed methods of measuring available carbon in soils and have correlated these measurements with measurements of soil "denitrification potential". For example, Stanford et al (1975) was able to show significant correlations between k the rate constant for denitrification, and total soil carbon (r^2=0.69) and glucose equivalent carbon extracted with 0.01 M $CaCl_2$ at 100°C for 1 hour (r^2=0.82) Burford and Bremner (1975) found that denitrification (appearance of N_2 and N_2O, or disappearance of NO_3^-) could be correlated with total soil carbon (r=0.77), water soluble soil carbon (r=0.99) and mineralizable soil carbon(r=0.99). These same parameters, with slight procedural modifications in some instances, were measured in the experimental soils of this study. It was hoped that these data would help

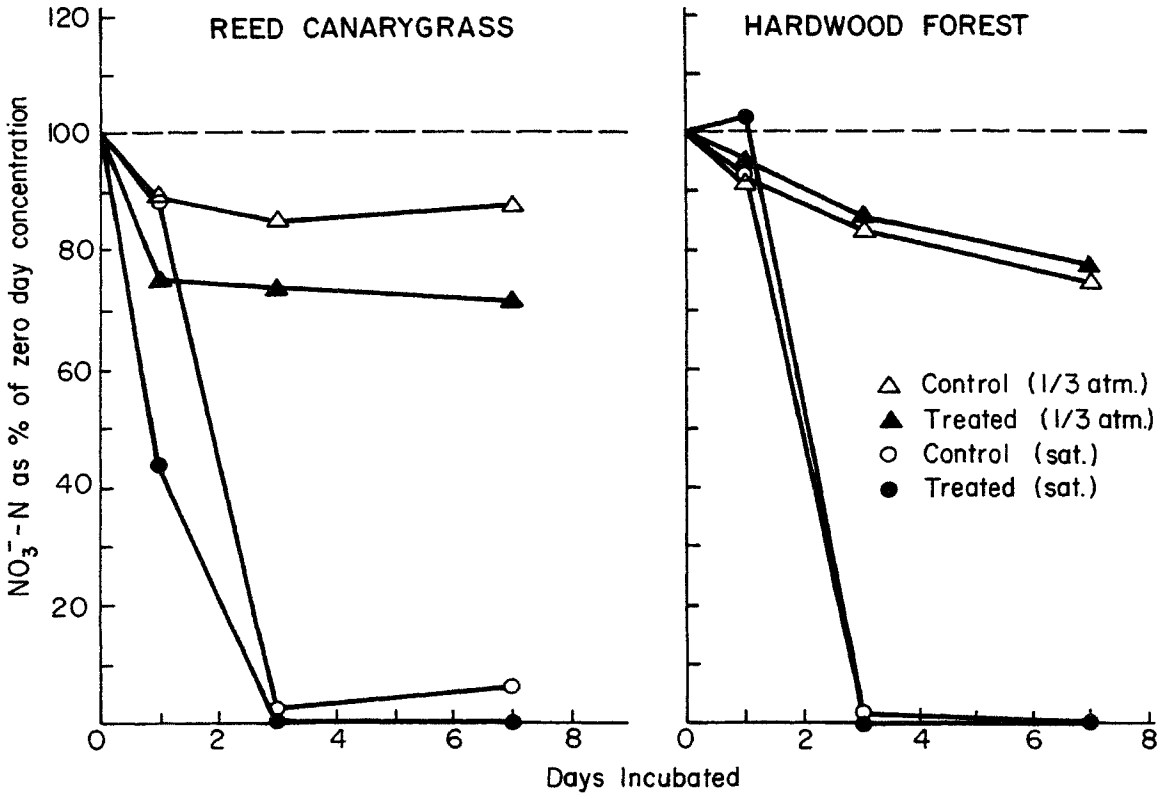

Figure 12. Effect of Glucose on the Loss of NO_3^--N from Wastewater Treated and Control Reed Canarygrass and Hardwood Forest Soils Incubated under 1/3 Atm. and Saturated Moisture Conditions (0-15 cm Depth).

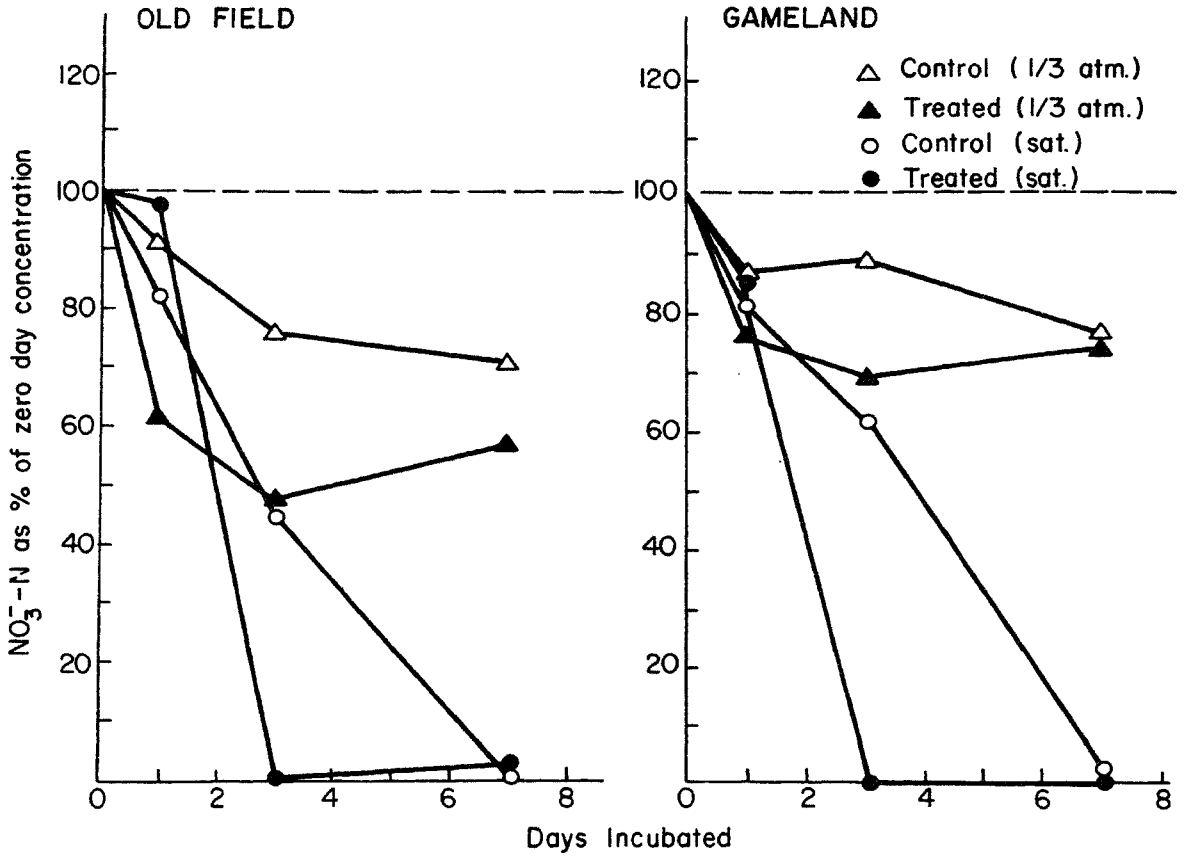

Figure 13. Effect of Glucose on the Loss of NO_3^--N from Wastewater Treated and Control Old Field and Gameland Soils Incubated Under 1/3 Atm. and Saturated Moisture Conditions (0-15 cm Depth).

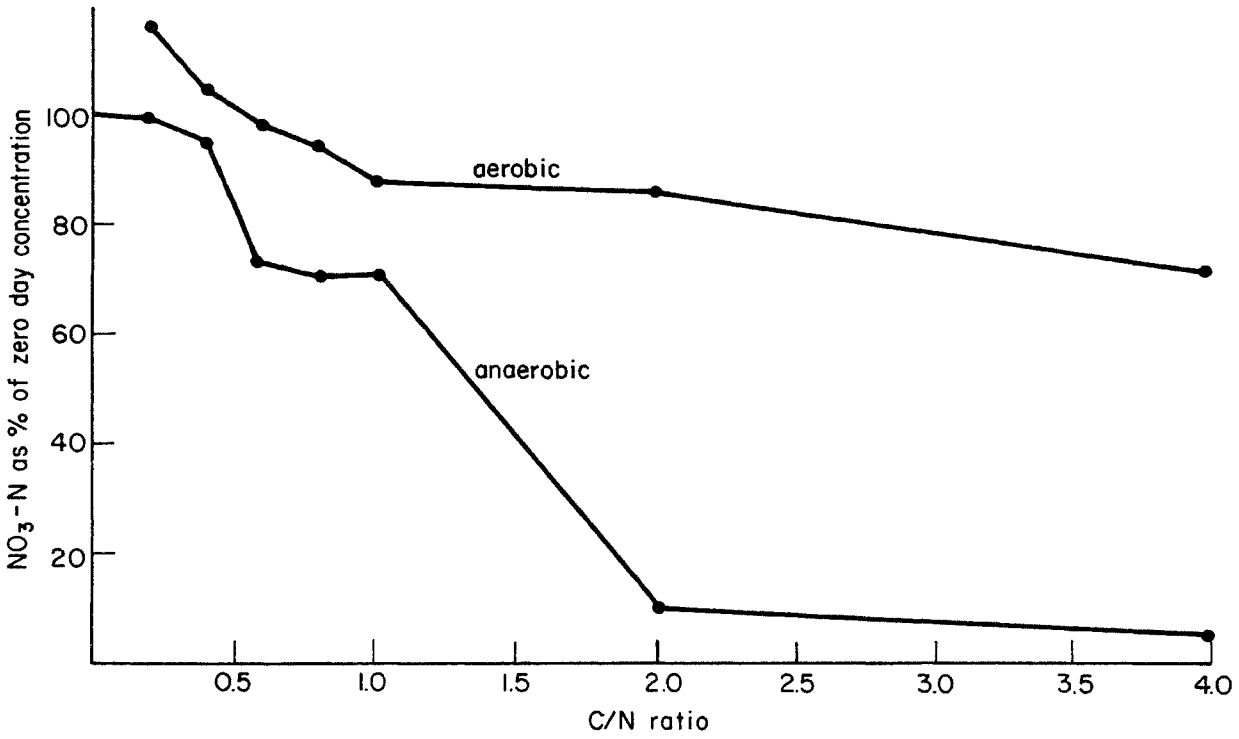

Figure 14. Effect of Differing C/N Ratio's on the Loss of NO_3^--N from Reed Canarygrass soil (0-15 cm Depth) after 7 Days Incubation Under 1/3 Atm. and Saturated Moisture Conditions.

explain the lack of denitrification found previously in these soils.

Data for soluble carbon and glucose equivalent carbon in soil samples from various depths from each of the soil vegetation systems for two sampling periods are shown in Table 8. Glucose equivalent carbon represents from 30-40% of the soluble carbon in the $CaCl_2$ extracts from the surface 0-7.5 and 7.5-15 cm layers and is the parameter directly comparable with the data of Stanford et al (1975). Glucose equivalent carbon for the 0-7.5 soil depth in all four wastewater management areas, both control and wastewater treated, were of near equal magnitude with soils which Stanford et al found to have reasonably high rates of denitrification (highest k values). Glucose equivalent carbon for the 7.5-15 cm depth were equal to soils which Stanford et al (1975) found to have the lowest rates of denitrification while the soils of the 15-30 cm and 30-60 cm depths had glucose equivalent carbon much lower than the reported values.

The discrepancy between the observations and data for the relationship between glucose equivalent carbon and denitrification in this study and that of Stanford et al (1975) cannot be explained from the available data. Perhaps differences in extractability, or denitrification occurred because the samples in this study were maintained at field moisture while those of Stanford et al (1975) had been air dried for about 7 years before their study was initiated. We do know, however, that the 0.01 M $CaCl_2$ extracts from the soils used in this study did not contain sufficient available carbon to support the growth of denitrifying bacterial isolates from these soils in liquid medium. Water soluble carbon and mineralizable carbon were also estimated on three of the wastewater treatment sites (Table 8 and 9). The soil samples were obtained in April at a time when soluble or labile organic matter should have been at its maxima. As expected soluble and mineralizable soil carbon decreased with depth in the profile. Generally, the wastewater treated samples were higher for both parameters of available carbon than control soils and at all depths. A comparison of these data with those obtained for 17 soils by Burford and Bremner (1975) indicates that even the surface soil samples (0-7.5 cm) of all three locations would fall in the lower range for supporting denitrification i.e. they had a low capacity for denitrification (again see Table 7). If it is assumed that the water soluble carbon found in this experiment (Table 9) was equivalent to glucose carbon and consideration is given to our previous statement based on Fig. 15 that about 40-50 µg of glucose carbon was necessary to initiate measurable denitrification, it follows that there was insufficient carbon to support denitrification in any of the soil samples. This certainly supports the previous measurements of the low"denitrification potential"in these same soils

The previous studies evaluated"denitrification potentials" of soil samples collected during a finite time period when plant residues from each vegetation area would have been at various and unknown stages of decomposition. It could be expected, for example, that samples collected in July, August, October and November would contain little residual plant material from the previous autumn-winter period. Thus all of the data discussed above did not provide answers for two questions, 1) does Reed Canarygrass leaf and thatch, weedy vegetation from the Old Field site, or leaf litter in the Forest Hardwood site support denitrification as they are decomposed in soil and, 2) how long would these residues if incorporated into the soil support increased denitrification before a return to background levels of denitrifying activity again are reached. These carbon compounds could be important in enhancing loss of residual NO_3^- in late autumn and early spring when the vegetation is not actively growing and utilizing NO_3^- . Carbon and nitrogen analyses for plant residues collected in autumn, 1975 are shown in Table 11.

Table 11. Carbon, Nitrogen Content, and C:N Ratio of Vegetation Recovered from Wastewater Renovation Site

Vegetation	Carbon	Nitrogen	C:N
	% (Dry Wt. Basis)		
Leaves and Thatch-Reed Canarygrass	35.3	3.2	11:1
Leaf litter**	46.5	1.0	46.5:1
Weedy-vegetation	42.6	0.9	47.3:1

* Most of the litter in the hardwood forest site was from Oak leaves.

In the first study, soil samples from the 0-15 cm depth of each of the wastewater treated and control areas was amended with 1% ground residues specific for each area and incubated for up to 9 weeks. Periodically during this incubation period, replicate soil samples were used to measure "denitrification potentials" at various stages of residue decomposition. The results of this study are shown in Table 12.

Data in Table 12 show that the added plant residues provide sufficient available organic carbon to support denitrification in the soils from the wastewater treated areas. Loss of over 70% of the added NO_3^- -N in 7 days occurred up to a 4 week decomposition period and significant losses of NO_3^--N could be measured for at least 9 weeks. Interestingly, denitrification activity in the control soils was consistantly less and for a shorter time period than the wastewater treated areas even though the same amount of residue was added to each soil sample. These data again follow very closely the relative population levels of denitrifying bacteria in treated and control soils estimated previously (See Fig. 8,9,10 & 11). Numbers of denitrifying bacteria as well as available carbon thus would limit denitrification in the control areas, but only available carbon in the more important wastewater treated areas.

The second study on the affect of residues on the denitrification potential was similar to the first except that an attempt was made to make the conditions more realistic. For example, the residues were cut into 1.5-2.5 cm sections rather than finely ground, the residue was incorporated in the surface 0-7.5 soil layer of 15 cm soil columns at a normal[1] loading rate of 0.6%, and the temperature of incubation was equivalent to the April, May, and June temperatures at the Penn State University Wastewater Management area. In addition, CO_2 evolution was measured concurrently in the residue treated soil samples incubated under saturated and 1/3 atm. moisture conditions.

The data for "denitrification potentials" in Table 13 follows the same trend as was shown previously in Table 12, although the magnitude of nitrate loss was considerably less than in the previous experiment. It seems likely that this reduction in nitrate loss could be the result of larger residue particle size in this second study which should reduce the microbial accesability of the residue to support denitrification. Maximum denitrifying activity was observed initially (0-day) and after 14 days residue composition. By one month the denitrification potential had been reduced about 50% and by 63 days to near control levels (no residue treatments). Maximum denitrifying activity coincided with that period of time where residue decomposition was proceeding slowly because of cool soil temperatures (Fig. 15 A, B, and C) and a pool of available substrate still remained. Conversely, the data of Fig 15 A,B, and C) also show a relationship between the ease of residue decompositon and denitrifying activity e.g. the "denitrification potential" and decompostion rate of the Reed Canarygrass residue at 14 and 29 days were lower than the Hardwood Forest or Old Field soils.

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[1] Estimates of residue present on the soil surface of all three wastewater management areas was equivalent to a 0.6% amendment (See Materials and Methods for details).

Table 12. "Denitrification Potential" of Soils from the Wastewater Treatment and Control Areas at Various Stages of Residue Decomposition (Flask Studies)

Soil-Vegetation System	Weeks of Preincubation						
	1	2	3	4	6	7	9
	% loss of added NO_3-N*						
Reed Canarygrass							
Treated	83.4	87.4	82.8	72.3	43.0	43.7	15.2
Control	82.6	61.5	52.0	44.9	10.0	2.1	0.0
Old Field							
Treated	92.2	95.5	82.6	78.0	48.3	36.4	24.2
Control	42.6	42.2	32.0	29.1	21.3	14.4	9.1
Hardwood Forest							
Treated	87.8	92.7	80.6	72.3	45.8	40.0	23.1
Control	42.6	42.2	32.0	29.1	21.3	14.4	9.1
Gameland							
Treated	95.8	98.5	95.1	83.2	65.9	60.0	23.3
Control	36.4	50.5	50.5	73.3	18.1	12.4	0.0

*Soil samples after each preincubation period were amended with 100 μg/g NO_3^--N and incubated under anaerobic conditions for 7 days.

Tabel 13. "Denitrification Potential" of Soils From the Wastewater Treatment Areas at Various Stages of Residue Decomposition (Soil Column Study)

Soil Treatments*	Days of Incubation 0	14	29	63	93
	% loss of added NO_3-N **				
CG-A-Sat.	48.0	31.2	20.4	8.5	0.0
CG-U-Sat.	10.0	12.0	4.4	2.2	0.0
CG-A-1/3 atm.	48.0	18.5	11.3	5.6	0.0
CG-U-1/3 atm.	10.0	5.0	4.2	3.4	0.0
OF-A-Sat.	53.5	55.0	29.3	9.5	0.0
OF-U-Sat.	29.0	36.5	18.5	0.9	0.0
OF-A-1/3 atm.	53.5	52.0	30.4	7.0	0.0
OF-U-1/3 atm.	29.0	22.5	8.8	0.4	0.0
HF-A-Sat.	37.0	56.0	25.0	4.5	0.0
HF-U-Sat.	6.5	9.5	3.7	4.4	0.0
HF-A-1/3 atm.	37.0	69.0	20.8	13.8	1.0
HF-U-1/3 atm.	6.5	2.5	8.6	1.1	0.0
Temp.	3° Night-17° C Day		8°-20°C		13°-25°C

* CG, OF, and HF refer to Canarygrass, Old Field and Hardwood Forest areas, U & A refer to unamended and residue amended treatments and Sat. + 1/3 atm. refers to the soil moisture content.

** Soil samples from the 0-7.5 cm layer of duplicate columns was removed at each indicated time period, amended with 100 µg/g NO_3^--N and incubated under anaerobic conditions for 5 days.

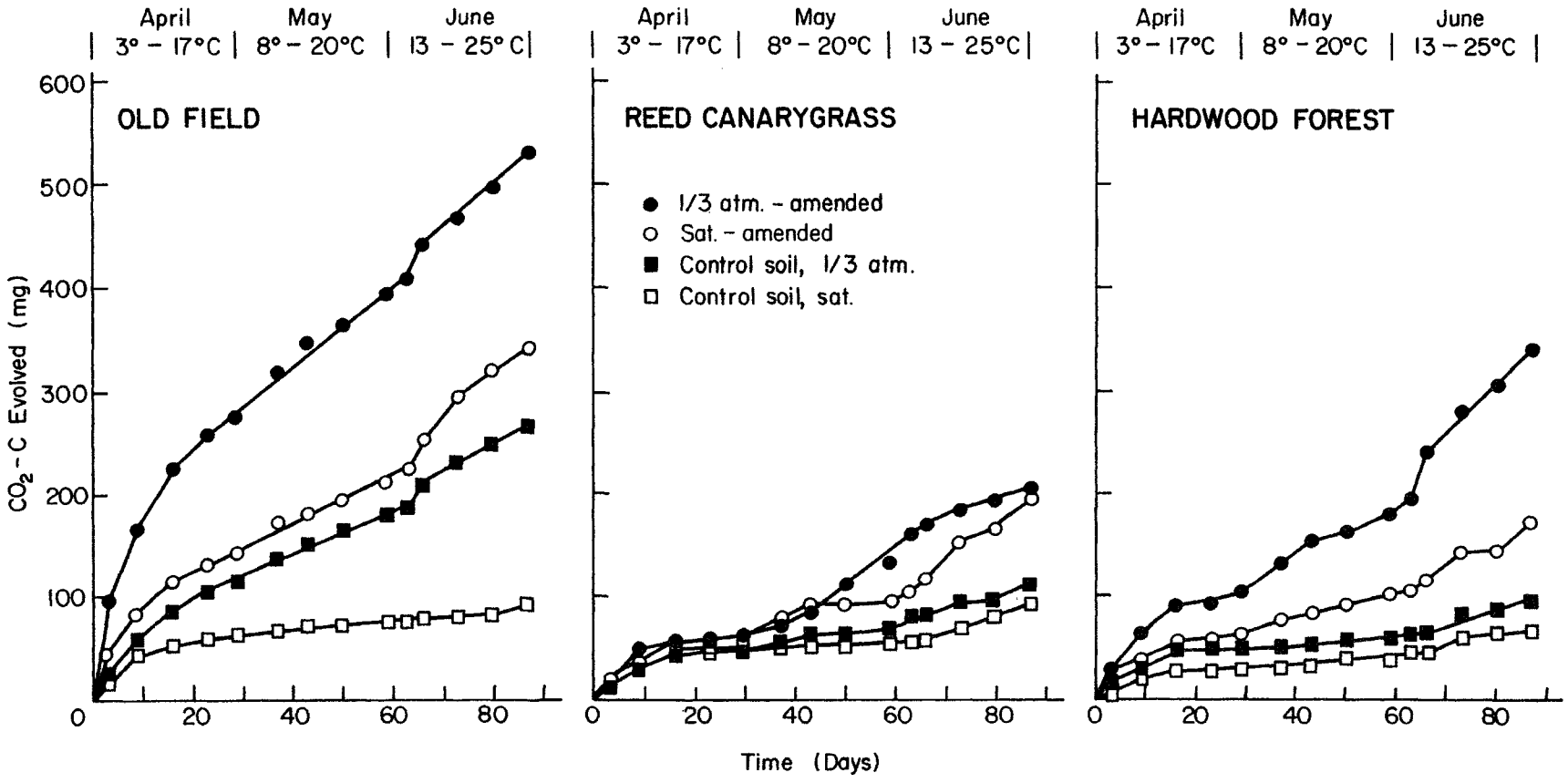

Figure 15. Cumulative CO_2-C Evolved From Residue Amended and Unamended Wastewater Treated Soil at 1/3 Atm. and Saturated Soil Moisture and Different Seasonal Temperatures.

Two other aspects of this study are worth noting. First, and surprisingly there was little difference in denitrifying activity in soils where the residues decomposed under saturated conditions vs 1/3 atm. moisture tension. Second, unlike the previous denitrification data of this study, the unamended soils did exhibit low levels of denitrifying activity.

Finally, the possible accumulation of substances toxic to denitrifying bacteria was investigated. Soils were extracted with water to remove possible water soluble toxic substances and the extracted soils were used to measure "denitrification potentials" after extraction. All of the extracted soils, except the Hardwood Forest soil, now had measurable and significant denitrifying activity, compared to activity before water extraction (compare the data in Figs. 16 and 17 with Table 7. There was, however, no appreciable differences between control and wastewater treated soils, which argues against any toxic substances associated with the wastewater application. These data suggest that there could be a general inhibitory substance of denitrification in all of the soils of both the control and wastewater treated sites. One other explanation is that improved denitrification in water-extracted soils was caused by the release of easily decomposable carbon after air drying of these extracted soils.

All of the above data demonstrated quite conclusively that the soils of the Penn State Wastewater Management areas contain insufficient carbon to support biological denitrification. These results were not particularly surprising for sub-surface zone, i.e. below 7.5 cm. However, the lack of denitrification in the surface 0-7.5 cm zone was totally unexpected. Annual additions of residue probably enhance denitrification during the spring before plant growth commences, however the effect will be of short duration and of limited value to total nitrogen renovation. The only NO_3^- likely to remain in the soil surface zone at this time would be that mineralized during April. Nitrate remaining from the previous years applications of wastewater would probably have leached below this soil zone. These data support the conclusion that empirical estimates of denitrification in soils receiving wastewater by spray irrigation probably have overestimated its significance. It is important that nitrogen loading rates in wastewater application sites be reduced to prevent the potential for groundwater contamination.

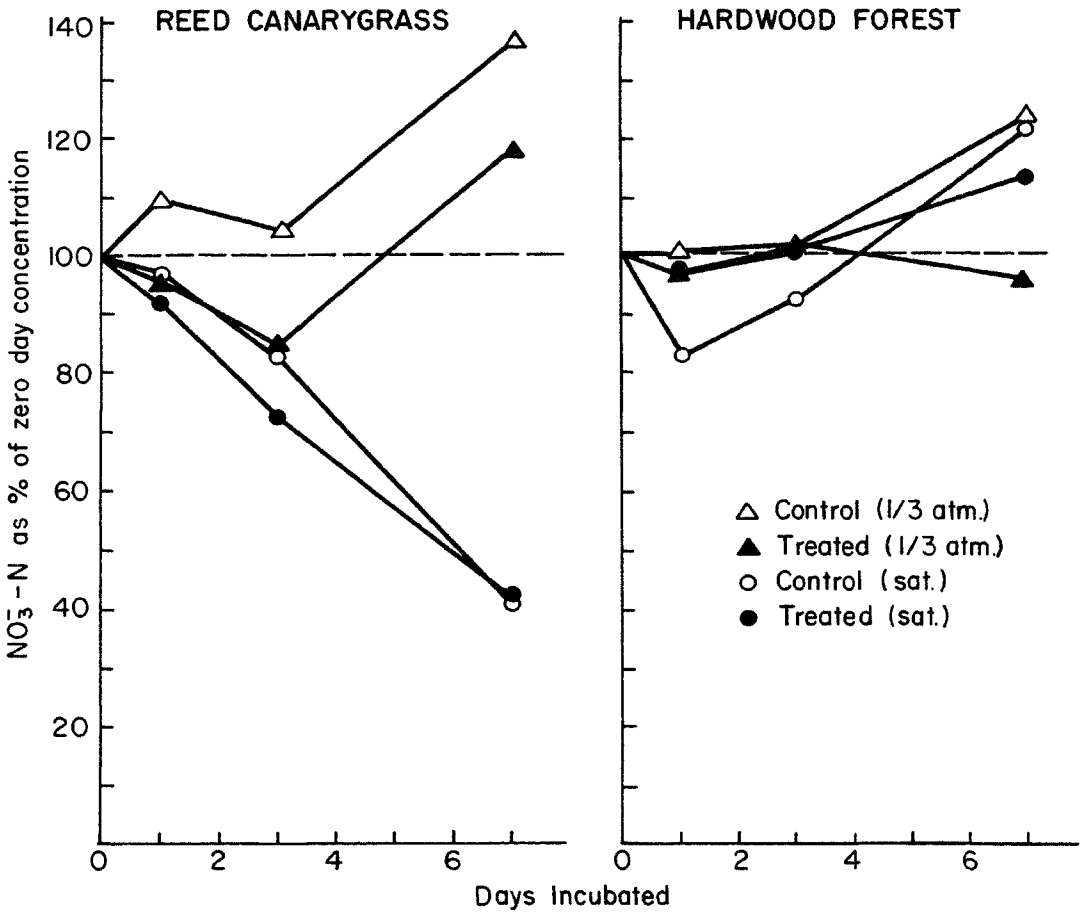

Figure 16. Loss of NO_3^--N From Wastewater Treated and Control Reed Canarygrass and Hardwood Forest Soils (0-15 cm Depth) after Water Extraction. The Extracted Soils Were Incubated Under 1/3 Atm. and Saturated Moisture Conditions.

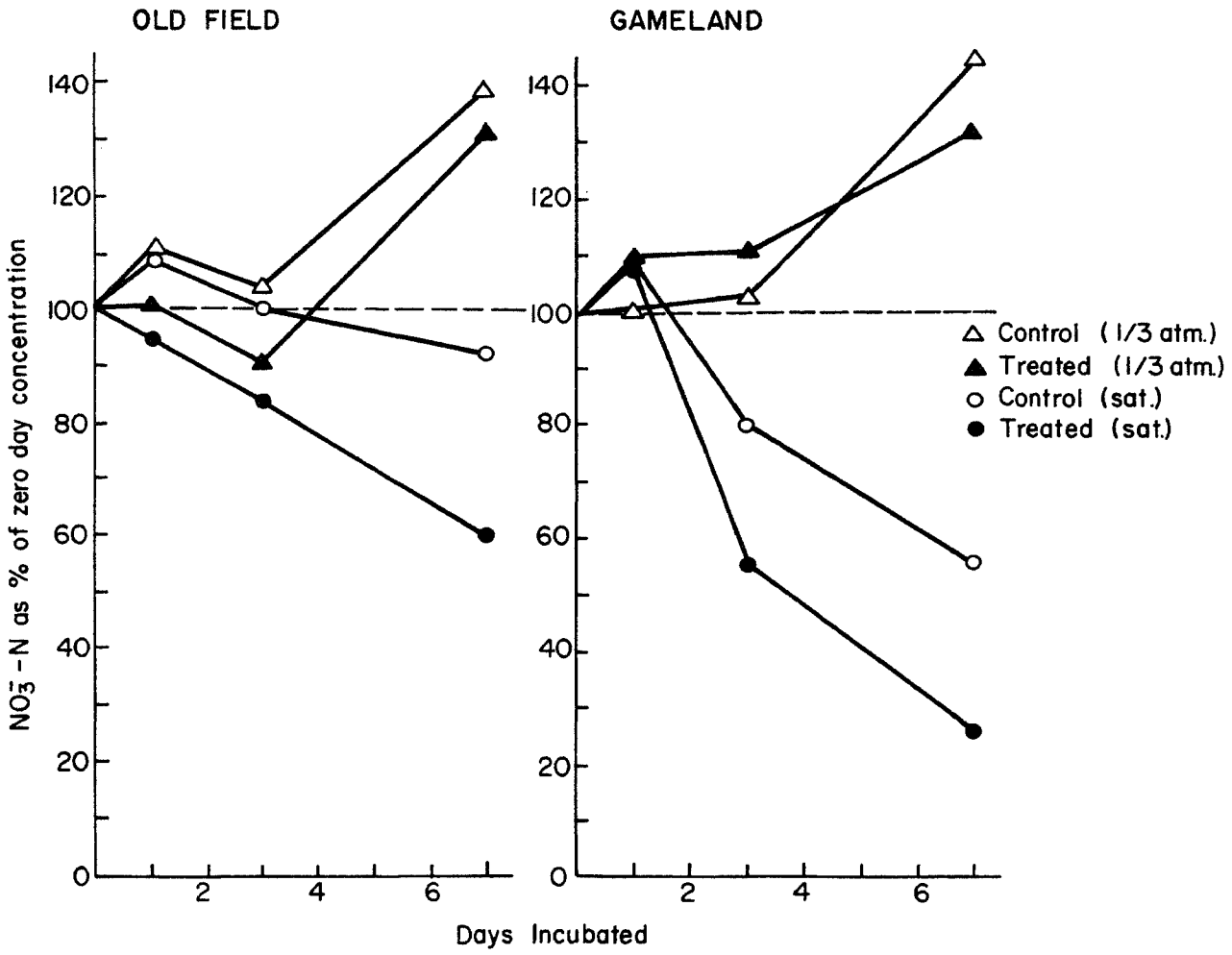

Figure 17. Loss of NO_3^--N From Wastewater Treated and Control Old Field and Gameland Soils (0-15 cm Depth) after Water Extraction. The Extracted Soils Were Incubated Under 1/3 Atm. and Saturated Moisture Conditions.

Studies of Nitrogen Renovation in Undisturbed Soil Cores

The studies discussed in the previous section showed that the soils from the Penn State University Wastewater Management areas contained insufficient available carbon to support biological denitrification. This was true even in the surface 7.5 cm depth. One possible source of carbon not investigated in these studies was carbon in the plant rhizosphere arising from root exudates or plant root cells sloughed during the growing season. A positive enhancement of denitrification in the rhizosphere has been shown by Waldendorp (1963), Brar (1972), Stefanson (1972) and Myers and McGarity (1972).

The studies described in this section utilized intact soil cores (10 cm x 15cm) from the Wastewater treated Reed Canarygrass area in an attempt to evaluate the potential rhizosphere influence on denitrification. It was hoped that denitrification losses could be estimated by measuring the difference between N input from a simulated effluent used to irrigate the Reed Canarygrass cores, N removed in the harvested vegetation, recovered in the leachate and present in the thatch, root tissue, and soil at the completion of the experiment . Data for N removed in the harvested top growth from five monthly harvests are given in Table 14 . The quantity of total NH_4^+ and NO_3^--N recovered in the leachate are also given in Table 14 and the pattern of N leaching in Fig. 18, 19, and 20. This experiment extended only 140 days, because a malfunction in the temperature control of the growth chamber resulted in the desication and killing of the established Reed Canarygrass. Although the experiment continued for an additional 60 days the resulting data was too erratic for further consideration.

The data presented in Table14 show the plant yield response to the N of the simulated effluent at all five monthly harvests. During the first three monthly havest periods the harvested Reed Canarygrass accounted for between 82.8 and 97.5% of the N added with the effluent. After this time the nitrogen content of the tissue as well as the yield decreased markedly and the amount of the applied N recovered also decreased. Reduced plant growth and N uptake was caused by increasing time of soil saturation as soil hydraulic conductivity declined. The harvested portion of the Reed Canarygrass accounted for 67.3% and 71.1% of the nitrogen applied at the 2.5 and 5.0 cm/week application rates over the 140 day period. Plant uptake thus represented the single most important factor in wastewater N renovation in the soil cores and was comparable in magnitude to that of the field studies on the same site (Sopper and Kardos, 1973).

Losses of nitrogen in the leachate were directly related to the rate of wastewater application and to the length of the experimental period (Table 15 and Fig. 18, 19, and 20).As expected most of the N found in the leachage was NO_3^-. The quantity of applied wastewater N in the leachate varied from 0 to 17.3% with a median value of about 6.0-7.0% for most of the study. The trend for decreased leachate N losses was distinct and significant and can be related to reduced soil hydraulic conductivity with time. The data on % recovery of added N provided in Table 15 suggests that increased denitrification losses after the 3rd monthly period were responsible for the improved leachate quality. The evidence for this interpretation is based on the drastic change in the quantity of applied N accounted for in the harvested plant material and the leachate. Note that during the first 3 months almost all of this applied N could be accounted for by these two parameters. After this,reduced soil hydraulic conductivity resulted in reduced plant vigor and a concomitant increase in available soil carbon for denitrification by exudation and root degradation. At the same time, the excellent recovery of applied

Table 14. Nitrogen Present in Harvested Reed Canarygrass and Leachate from Intact Soil Cores

Harvest Time	Treatment	Grass Yield g/core	N %	N Recovered mg/core	% of added N*	N in Leachate mg/core	% of added N	Total Recovery %
	CONTROL	1.021	2.78	28.4	-	1.093	-	-
28 days	2.5 cm	1.464	3.80	41.0	97.5	1.955	6.7	104.2
	5.0 cm	1.625	3.13	50.9	87.1	5.572	17.3	104.4
	CONTROL	0.990	2.49	24.7	-	0.452	-	-
56 days	2.5 cm	1.427	2.52	36.0	87.5	1.394	7.3	94.8
	5.0 cm	1.683	2.88	48.5	92.1	1.920	5.7	97.8
	CONTROL	0.963	2.16	20.8	-	0.485	-	-
84 days	2.5 cm	1.527	2.06	31.5	82.8	1.393	7.0	89.8
	5.0 cm	2.003	2.16	43.3	87.1	1.774	5.0	92.1
	CONTROL	0.675	2.28	15.4	-	0.473	-	-
112 days	2.5 cm	1.026	2.28	23.4	61.9	0.777	2.4	64.3
	5.0 cm	1.617	2.13	34.4	73.5	0.980	2.0	75.5
	CONTROL	0.401	2.24	9.0	-	0.077	-	-
140 days	2.5 cm	0.446	2.22	9.9	7.0	0.048	0	7.0
	5.0 cm	0.598	2.19	13.1	15.9	0.067	0	15.9

* Nitrogen added with the simulated effluent was 12.9/wk mg at the 2.5 cm rate and 25.8 mg/wk at the 5.0 cm rate.

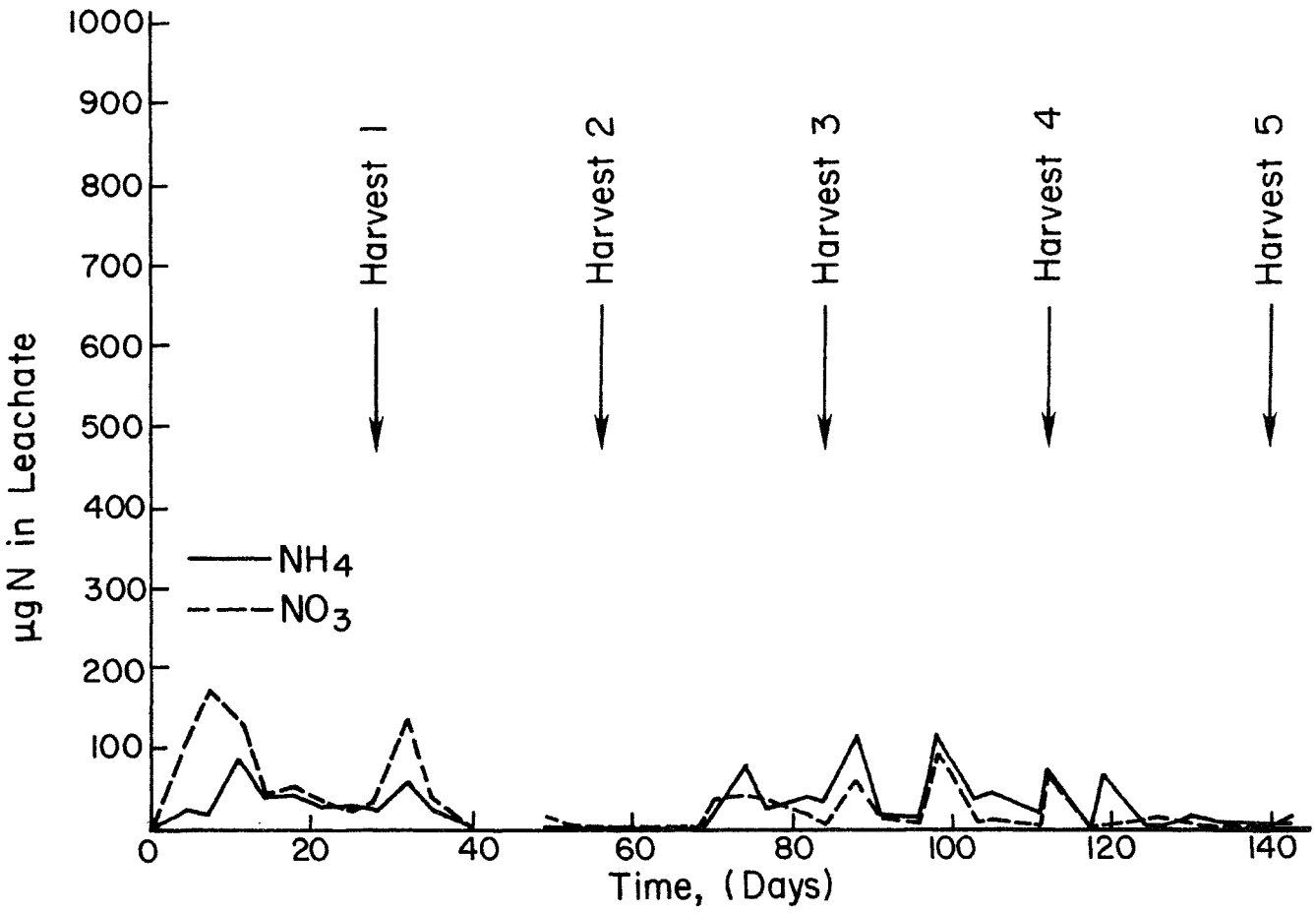

Figure 18. Concentration of NH_4^+ and NO_3^--N in the Leachate from Intact 15 cm Soil Cores From the Wastewater Treated Reed Canarygrass Site (No Applied Effluent).

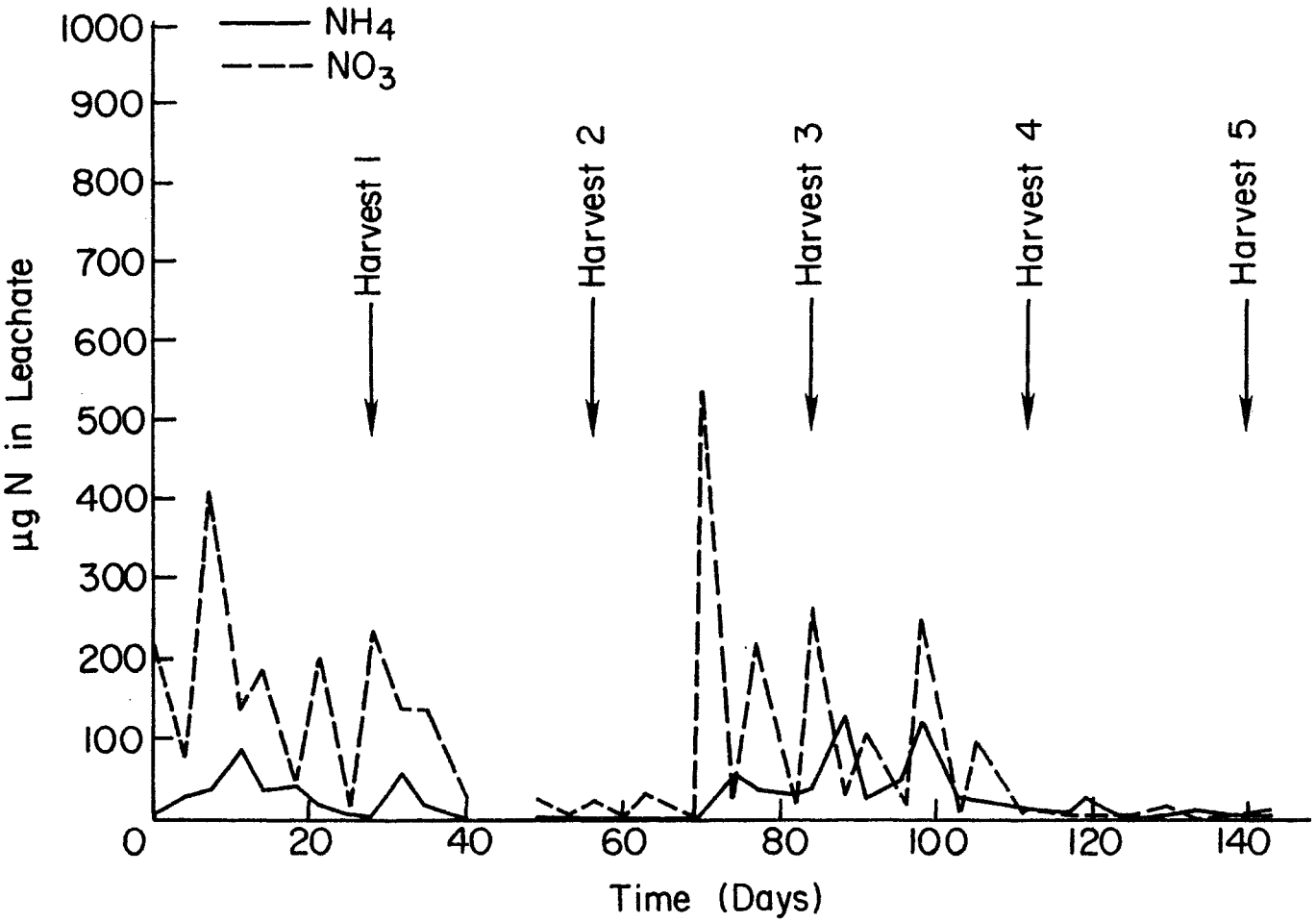

Figure 19. Concentration of NH_4^+ and NO_3^--N in The Leachate from Intact 15 cm Soil Cores From the Wastewater Treated Reed Canarygrass Site (2.5 cm Effluent/wk.).

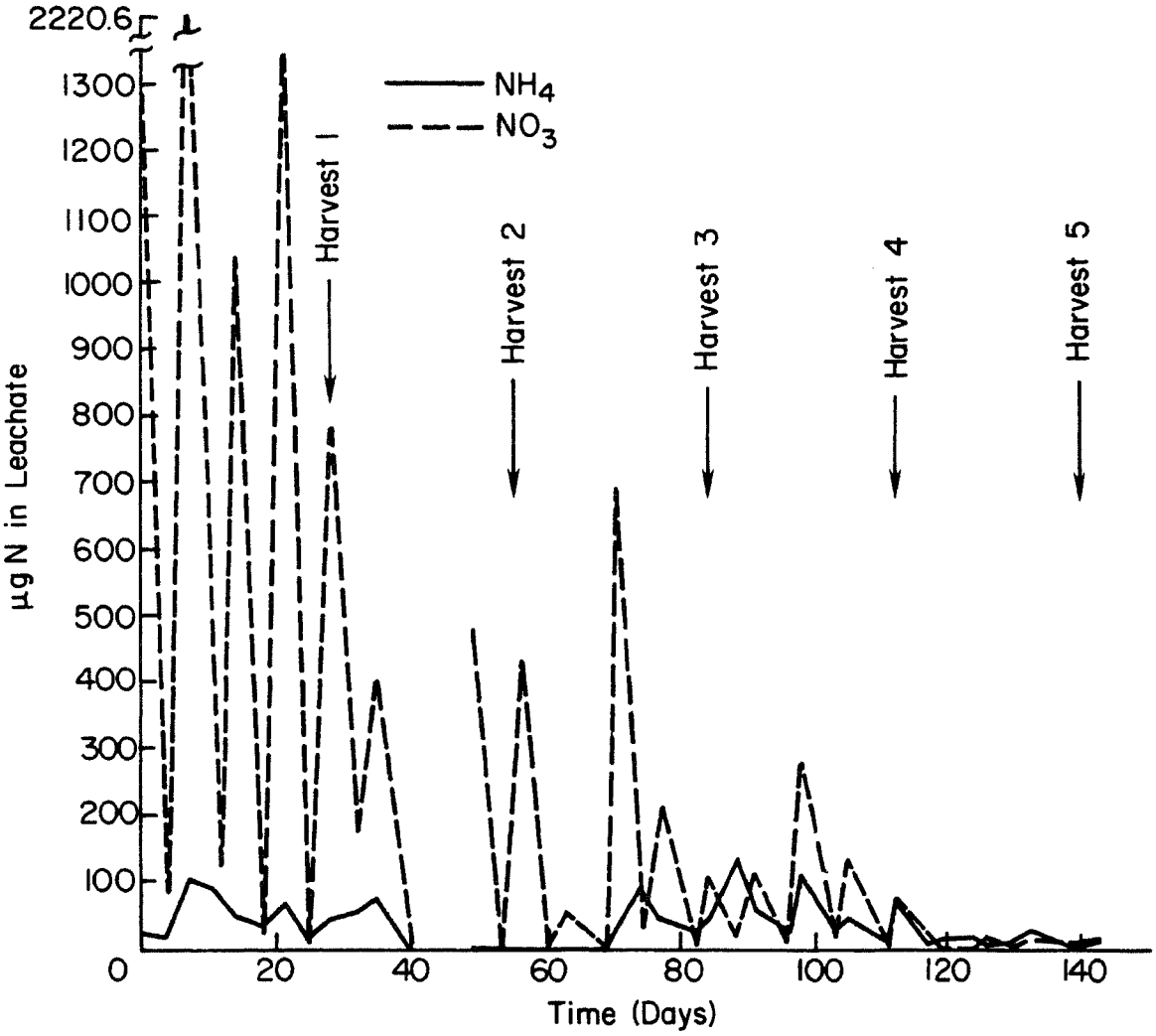

Figure 20 Concentration of NH_4^+ and NO_3^--N in The Leachate from Intact 15 cm Soil Cores from the Wastewater Treated Reed Canarygrass Site (5.0 cm Effluent/wk.).

N during the first 3 months of the study suggest that rhizosphere root exudates from healthy actively growing plants does not support biological denitrification even though the soils were saturated for periods as long as 12-18 hours.

Finally, residual N in the stubble and thatch, and in the root + soil portion of the soil cores at the completion of the experiment differed very little between the wastewater treated and control cores. These data support the previous comment that some denitrification occurred in these soils during the last 2 months of the study.

The overall data from the soil core study does not alter the previous conclusion that the soils of the Penn State Wastewater Management area do not support biological denitrification. Reed Canarygrass did not provide sufficient organic carbon in the rhizosphere under normal growth conditions to support an appreciable loss of NO_3^-. Only under extreme conditions of soil waterlogging was denitrification quantitatively significant

Seasonal Changes in Root Mass in the Reed Canarygrass Area

Data has accumulated on the amount of nitrogen removed from the Reed Canarygrass area with the harvested forage (Kardos, et al, 1974). Their data report an average annual removal of 336 lbs/acre (375 kg/ha) of nitrogen during 1965-69 with forage yields of 5.55 T/acre. This removal was equivalent to ~ 70% of the nitrogen added with a 5 cm/week application of effluent.

No information is available concerning the mass and N content of the root tissue of Reed Canarygrass during wastewater application, or if seasonal changes occur in the quantity of root tissue or combined nitrogen. These data are significant for calculating an N balance for soils receiving wastewater, because considerable applied N could be immobilized during periods of accumulation of root mass or release from dead and decaying roots during seasonal decreases in root mass. For example, Weaver and Zink (1946) in a study of 10 species of perennial range and pasture grasses estimate that roots live 2 to 3 years (a 33-50% annual turnover) while Dahlman and Kucera (1965) estimate that 25% of the root system of prarie grasses will be replaced each year. In addition to nitrogen cycling, such changes in root mass could provide an important periodic source of energy for denitrifying bacteria.

Measurements were made of total root mass and root organic matter, organic carbon, and organic nitrogen in the wastewater treated Reed Canarygrass area during 1974-75. These data are shown in Fig. 21 and Table 15. Definite seasonal changes in all parameters were evident with a maximum in summer during the period of maximum growth and a decrease to a minimum in spring before active growth had begun. The maximum root mass in early August, 1975 was higher than late July, 1974. No explanation for this difference can be made but it may reflect differences in the growth environment, such as difference in mean light intensity, rainfall, or temperature.

The decrease in root mass, organic matter and organic carbon from July till April would indicate a root turnover rate of about 44-48%. This turnover rate would be in agreement with that proposed by Weaver and Zink (1946) noted earlier. The total root carbon available for microbial decomposition during this period would be about 2920 kg/ha.

The change in root organic nitrogen from July to April is much less and represents only about a 25% decrease. These data suggest a plant mechanism for conserving nitrogen in autumn and early spring as soil nitrogen additions are no longer being made and plant growth demands also decrease. Perhaps nitrogen can be mobilized and translocated to the remaining viable root tissue with a lowered C/N ratio as shown in Table 15. At the same time the approximate C/N ratio for the dead root tissue is 57/1, a ratio which would result in nitrogen immobilization as the root residue is decomposed and humified.

Table 15. Seasonal Changes in Root Mass and Associated Parameters In Wastewater Treated Reed Canarygrass

Sampling Date	Root				%	
	Mass	Organic Matter	Organic C	Organic N	Organic Matter	C/N
	kg/ha					
July, 74	15,356	10191	4076	159	66.4	25.6
Oct.,74	13,040	8657	3463	143	66.4	24.3
April, 75	6,686	4889	1956	121	73.1	16.2
Aug., 75	18,347	12535	5014	252	68.8	19.9

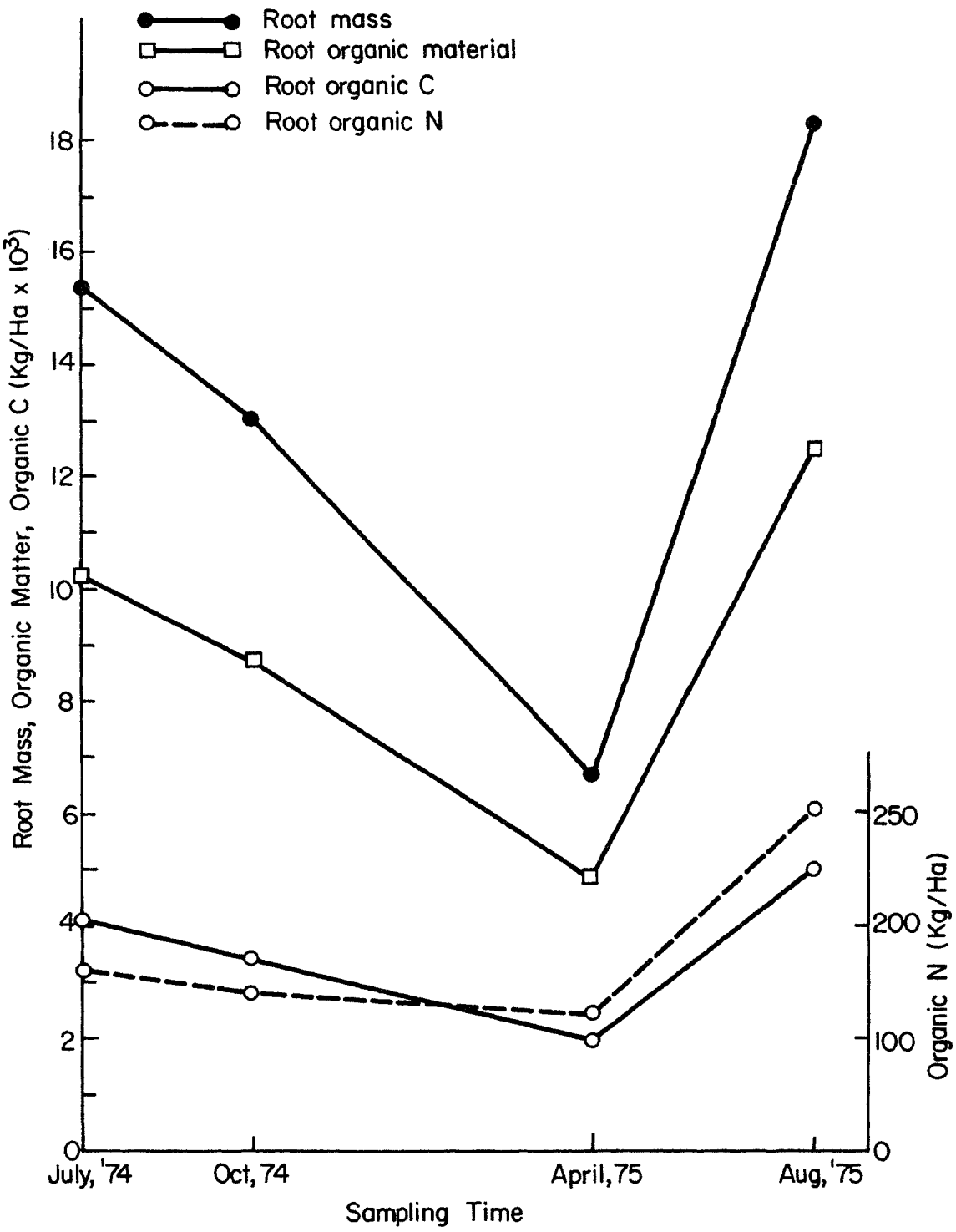

Figure 21. Seasonal Changes in Root Mass. Organic Matter, Organic Carbon and Nitrogen in Wastewater Treated Reed Canarygrass.

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Reed Canarygrass site (RCG)

RCG 1 - between irrigation lines 8 and 9 and adjacent to piezometer site 2.

RCG 2 - between irrigation lines 8 and 9 and adjacent to piezometer site 4.

RCG 3 - between irrigation lines 8 and 9 and adjacent to piezometer site 5.

RCG 4 - Control. Sampled in center of control area.

Hardwood forest site (FH)

FH 1 - just inside forest from the road on the left side of the trail and adjacent to lysimeter.

FH 2 - just downslope from sink hole and adjacent to runoff pans.

FH 3 - further downslope and adjacent to last irrigation riser.

FH 4 - Control. Right side of trail and across from last irrigation riser. Next to animal burrow.

Old Field site (OF)

OF 1 - On left side of trail between irrigation lines. Adjacent to runoff pan.

OF 2 - On right side of trail between irrigation lines. Just to right of second lysimeter.

OF 3 - On right side of trail between irrigation lines. Adjacent to lysimeter 20 and between two large fir trees.

OF 4 - Control. Just below lysimeter 24.

Gamelands area (FM)

FM 1 - adjacent to first irrigation line and on side nearest road.

FM 2 - adjacent to first irrigation line and on side away from road.

FM 3 - same side as FM 1 but to left of FM 1 with respect to the trail.

FM 4 - Control. To the right of the treated area with respect to the trail and as far in from the road as the other sites.

Appendix B . Composition of Simulated Secondary Effluent

Nutrient	mg/l	Salt Used	Formula weight	Amount used mg/l
$PO_4^{=}$-P	5	Na $H_2PO_4.H_2O$	138	22.25
NO_3^{-}-N	15	Na NO_3	85	91.07
NH_4^{+}-N	4	$(NH_4)_2SO_4$	132	18.85
K^{+}	8	KCl	74.5	15.28
Mg^{2+}	20	Mg $Cl_2.6H_2O$	203	84.50
		Mg CO_3	84	35.00
Fe^{2+}	2.5	$FeSO_4.7H_2O$	278	12.46
Na^{+}	40	Na_2CO_3	106	13.80
		Na HCO_3	84	21.90
Ca	34	$CaCl_2.6H_2O$	147	20.250
		$CaCO_3$	100	81.750

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16. Abstract

Nitrogen transformations on soils of the Pennsylvania State University Wastewater Management Study were investigated for Reed Canarygrass, Old Field, Hardwood Forest and Gameland sites. Nitrogen mineralization rates, plotted as N mineralized versus $t^{\frac{1}{2}}$ for the 26 week mineralization period were linear for 0-7.5 cm soil depths for all sites, but only for eight weeks at the 7.5-15 cm depth, reflecting the depletion of mineralizable N compounds at lower depths. N mineralization was higher for wastewater treated sites on Hublersberg soil and represented 3.0-3.5% of the soil N; rates were 5-9 times greater on the coarse-textured Morrison soil, indicating a greater danger of NO_3^- leaching with wastewater treatment of this type of soil. Nitrification was rapid on all treated soils, absent on Old Field and Gameland control sites and slow on Hardwood Forest sites due to low soil pH. Denitrifying activity was low on all sites even in surface layers; glucose-amended soils, however, gave rapid denitrification, indicating a lack of available carbon. This was supported by measurements of soluble and mineralizable carbon in soil. Plant residues taken from the site supplied available carbon for denitrification for 2-4 weeks only.

17a. Descriptors

nitrification*, denitrification*, waste water disposal*, soil microbiology, water pollution, sewage effluents.

17b. Identifiers

Pennsylvania State University Wastewater Management Study*, nitrogen mineralization, available soil carbon, plant residue.

17c. COWRR Field & Group 05E

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